Pesticide residues in food – 2009

Joint FAO/WHO Meeting on Pesticide Residues

EVALUATIONS 2009

Part II — Toxicological

Food and Agriculture Organization of the United Nations

World Health Organization

Pesticide residues in food — 2009

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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2009 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

Geneva, 16–25 September 2009

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Abbreviations used

2-AAF	2-acetylaminofluorene
ACC	acetyl coenzyme A carboxylase
AChE	acetylcholinesterase
AD	administered dose
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and elimination
A/G	albumin to globulin ratio
ai	active ingredient
ALAT	alanine aminotransferase
ALD	aldrin epoxidase
AP	alkaline phosphatase
APTT	activated partial thromboplastin time
ARfD	acute reference dose
ASAT	aspartate aminotransferase
AUC	area under the curve
$AUC_{(0-\infty)}$	area under the curve from time zero to infinity
ax.	axial isomer
BrdU	bromodeoxyuridine
BROD	benzoxyresorufin *O*-debenzylase
BUN	blood urea nitrogen
bw	body weight
cAMP	cyclic adenosine monophosphate
CAR	constitutive androstane receptor
CAS	Chemical Abstracts Service
ChE	cholinesterase
CHO	Chinese hamster ovary
CL	clearance rate
C_{max}	peak concentration in blood
CMC	carboxymethyl cellulose
CoA	coenzyme A
CPP	cyclophosphamide
CYP	cytochrome P450
db-cAMP	dibutyryl-cyclic adenosine monophosphate
DMNA	dimethylnitrosamine
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTNB	5,5′-dithiobis(2-nitrobenzoic) acid
ECOD	7-ethoxycoumarin deethylase

ED_{30}	dose associated with a 30% decrease in motor activity
EEC	European Economic Community
EH	epoxide hydrolase
EMS	ethylmethanesulfonate
eq.	equatorial isomer
equiv.	equivalents
EROD	7-ethoxyresorufin *O*-deethylase
F	female
FAH	foci of altered hepatocytes
FAO	Food and Agriculture Organization of the United Nations
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act (USA)
FOB	functional observational battery
GC	gas chromatography
GD	gestation day
GGT	γ-glutamyl transferase
GLDH	glutamate dehydrogenase
GLP	good laboratory practice
GLUT	UDP-glucuronyltransferase
GPT	glutamic–pyruvic transaminase
GST	glutathione *S*-transferase
Hb	haemoglobin
hCG	human chorionic gonadotrophin
H&E	haematoxylin and eosin
HID	highest ineffective dose
HPLC	high-performance liquid chromatography
Ht	haematocrit
IC_{50}	median inhibitory concentration
Ig	immunoglobulin
i.p.	intraperitoneal
IPCS	International Programme on Chemical Safety
ISO	International Organization for Standardization
IU	international units
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
k_i	inhibition rate constant
LAH	lauric acid hydroxylase
LC	liquid chromatography
LC_{50}	median inhibitory concentration
LD	lactation day
LD_{50}	median inhibitory dose
LED	lowest effective dose

LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
LS	longitudinal section
M	male
MCA	methylcholanthrene
MCH	mean corpuscular haemoglobin; mean cell haemoglobin
MCHC	mean cell haemoglobin concentration
MCV	mean corpuscular volume
MD	malate dehydrogenase
ME	malic enzyme
MMAD	mass median aerodynamic diameter
MOA	mode of action
MPA	2-methyl-3-phenylbenzoic acid
MRT	maximum residence time
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MTD	maximum tolerated dose
NA	not available; not applicable
NADH	reduced nicotinamide adenine dinucleotide
NADPH	reduced nicotinamide adenine dinucleotide phosphate
ND	not detected; not determined
N-DEM	aminopyrine-*N*-demethylase
NMR	nuclear magnetic resonance
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NR	not reported
n.s.	not statistically significant
NTE	neuropathy target esterase
NZW	New Zealand White
O-DEM	*p*-nitroanisole-*O*-deethylase
OECD	Organisation for Economic Co-operation and Development
OH	hydroxy
OPPTS	Office of Prevention, Pesticides and Toxic Substances (USEPA)
p.c.	postcoitum
PCNA	proliferating cell nuclear antigen
PFCA	plaque-forming cell assay
p.i.	post-insemination
PLP	pyridoxal-5′-phosphate
PND	postnatal day

p.o.	per os (by mouth)
p.p.	postpartum
ppb	parts per billion
ppm	parts per million
PROD	pentoxyresorufin *O*-dependentylase
PXR	pregnane X receptor
QA	quality assurance
RBC	red blood cell (erythrocyte)
RIP	relative inhibitory potency
roi	region of interest
ROLD	repeated oral low dose
S9	9000 × *g* rat liver supernatant
SD	standard deviation; Sprague-Dawley
SOHD	single oral high dose
SOLD	single oral low dose
T_3	triiodothyronine
T_4	thyroxine
TCP	3,5,6-trichloro-2-pyridinol
TEM	transmission electron microscopy
TFP	trifluoropropenyl
TLC	thin-layer chromatography
T_{max}	time to peak concentration in blood
TOCP	tri-*ortho*-cresyl phosphate
TOTP	tri-*ortho*-tolyl phosphate
TRR	total radioactive residue
TS	transverse section
TSH	thyroid stimulating hormone
U	unit
UDP	uridine diphosphate
UDS	unscheduled DNA synthesis
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	ultraviolet
v/v	by volume
Vz	volume of distribution
WHO	World Health Organization
w/v	weight per volume
w/w	by 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 16–25 September 2009.

Three of the substances evaluated by the WHO Core Assessment Group (fluopicolide, metaflumizone and spirodiclofen) were evaluated for the first time. Five compounds (bifenthrin, cadusafos, chlorothalonil, chlorpyrifos-methyl and cycloxydim) were re-evaluated within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR). Reports and other documents resulting from previous Joint Meetings on Pesticide Residues are listed in Annex 1.

The report of the Joint Meeting has been published by the FAO as *FAO Plant Production and Protection Paper 196*. 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 2009, 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 2009 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

BIFENTHRIN

First draft prepared by
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Explanation

Bifenthrin is the International Organization for Standardization (ISO)–approved name for (2-methyl-3-phenylphenyl) methyl (1*RS*, 3*RS*)-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate (International Union of Pure and Applied Chemistry [IUPAC]), for which the Chemical Abstracts Service (CAS) No. is 82657-04-3. Bifenthrin is a synthetic pyrethroid insecticide and acaricide.

The toxicity of bifenthrin was first evaluated by the 1992 Joint FAO/WHO Meeting on Pesticide Residues (JMPR). The Meeting established an acceptable daily intake (ADI) of 0–0.02 mg/kg body weight (bw) on the basis of a no-observed-adverse-effect level (NOAEL) of 1.5 mg/kg bw per day for decreased body weight gain in males and dose-related tremors in a 1-year study of oral toxicity in dogs and with a safety factor of 100.

New studies of acute and dermal toxicity, sensitization, neurotoxicity, developmental toxicity and genotoxicity and a pathology re-evaluation of the tumours observed in the study of carcinogenicity in mice became available since the last review by JMPR. Bifenthrin was reviewed by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues. All pivotal studies with bifenthrin were certified as complying with good laboratory practice (GLP).

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 guideline(s).

1. Biochemical aspects

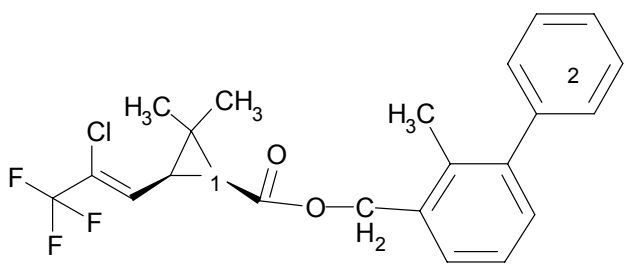
1.1 Absorption, distribution, metabolism and excretion

(a) Oral administration

Rats

The absorption, distribution and elimination of bifenthrin were studied after oral dosing of rats with bifenthrin radiolabelled with ^{14}C, as shown in Figure 1.

Figure 1. Position of the radiolabel on bifenthrin used in pharmacokinetic studies in rats



1: Acid (cyclopropyl) ^{14}C bifenthrin

2: Alcohol (phenyl) ^{14}C bifenthrin

The absorption, distribution and metabolism of bifenthrin labelled with ^{14}C in either the alcohol (phenyl) or acid (cyclopropyl) ring were studied in Sprague-Dawley rats (three rats of each sex per dose) following a single gavage dose of 5 mg/kg bw in corn oil. Treated animals were kept individually in metal metabolism cages for collection of urine and faeces. Samples of urine and faeces were collected at the following time intervals: 0–8, 8–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 h post-dosing. Treated rats were sacrificed after 7 days, and radioactivity in various tissues and organs was analysed. Samples of urine and faeces were extracted and analysed for metabolites using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Approximately 90.7% and 91.9% of the administered dose of alcohol-labelled bifenthrin were recovered in the excreta of male and female rats, respectively, in 7 days. Approximately 86.0% and 86.5% of the administered dose of acid-labelled bifenthrin were recovered in the excreta of male and female rats, respectively, in 7 days. Most of the orally administered dose (for both labels) was recovered in the excreta, predominantly in faeces, in the first 48 h following dosing. In the first 48 h, 76.2–79.5% and 72.9–74.0% of the administered dose were recovered in faeces and 5.6–6.9% and 8.4–9.2% in urine for the alcohol and acid labels, respectively. The highest level of radioactivity was detected in fat (1.7 and 0.8 parts per million [ppm] for alcohol- and acid-labelled bifenthrin, respectively) in 7 days. TLC analysis and comparison of the 0–48 h extractable faecal metabolites obtained from alcohol- and acid-labelled bifenthrin, for both male and female rats, showed identical numbers of metabolites, with intact unmetabolized parent compound predominating. The results suggested that the intact pyrethroid ester group was stable and that very minimal hydrolysis of both labels occurred. This preliminary study demonstrated that most of the orally administered dose of bifenthrin in rats was excreted in the first 48 h, predominantly in faeces, and there was no influence of sex or radiolabelling position of bifenthrin on the absorption, distribution, metabolism or elimination in rats. The analytical profile of extractable ^{14}C residues from both labels showed a similar chromatographic pattern, indicating no extensive esterase splitting activity on the parent compound (ElNaggar, 1987).

In toxicokinetic studies, groups of male and female Sprague-Dawley rats (five of each sex per dose) were given radiolabelled bifenthrin as a single oral dose at 4 or 35 mg/kg bw by gavage or as 14 repeated oral doses of 4 mg/kg bw per day followed by a single oral dose of radioactive bifenthrin at 4 mg/kg bw. All female rats received bifenthrin (purity 98%) labelled with ^{14}C in the alcohol (phenyl) ring, and all male rats were dosed with bifenthrin (purity 97.3%) labelled with ^{14}C in the acid (cyclopropyl) ring. Treated rats were housed in stainless steel metabolism cages for 7 days. Urine and faeces were collected at the following time intervals: 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–72, 72–96, 96–120, 120–144 and 144–168 h. At each collection, cages were rinsed with water and cage washings collected together with the urine. At the end of 7 days, animals were sacrificed, and blood, tissues and organs were analysed for radioactivity. In a separate experiment, two male and two female rats were dosed with a single gavage dose of radiolabelled bifenthrin at 4 mg/kg bw and housed in Roth metabolism cages for collection of expired air and organic volatiles using a solution of 2:1 ethanolamine:Cellosolve at intervals of 4, 8, 12, 24 and 48 h.

Clinical signs of toxicity and mortality were observed in the 35 mg/kg bw dosed group but not at the 4 mg/kg bw single dose or repeated dose. The amounts of radioactivity recovered in the expired air and volatile trap were 0.028% and 0.053% of the administered dose in females and males, respectively, in 48 h. Organic volatiles were not collected because a preliminary study indicated a small quantity of radioactivity in this fraction. A majority of bifenthrin-derived radioactivity from the single oral low dose, single oral high dose and multiple oral low doses (acid or alcohol labelled) was excreted in faeces, amounting to 73–83%, 69–71% and 66–73% of the administered dose, respectively. Urine contained only 13–20%, 22% and 18–25% following the single oral low dose, single oral high dose and multiple oral low doses, respectively. Elimination of most ^{14}C-labelled bifenthrin

Table 1. Distribution of radioactivity recovered in rats following gavage dosing of bifenthrin at 168 h post-dosing

Dose (mg/kg bw)	Sex	Distribution of radioactivity (% of administered dose)			
		Urine	Faeces	Tissues	Total
4	Male[a]	13.39 (± 5.77)[c]	82.80 (± 8.85)	3.76 (± 2.65)	100.95 (± 6.86)
	Female[b]	19.65 (± 4.93)	72.87 (± 4.98)	3.94 (± 0.95)	96.46 (± 1.43)
35	Male	21.60 (± 7.93)	68.89 (± 6.64)	3.40 (± 1.83)	93.90 (± 5.19)
	Female	21.76 (± 1.85)	70.93 (± 5.69)	4.22 (± 1.93)	96.92 (± 5.64)
4 (repeated dose)	Male	18.36 (± 3.58)	73.22 (± 4.82)	3.13 (± 1.09)	94.71 (± 3.98)
	Female	25.01 (± 7.26)	65.80 (± 9.60)	4.05 (± 1.27)	94.85 (± 4.69)

Data extracted from Selim (1987), Table 28-33

[a] Males were dosed with bifenthrin labelled with ^{14}C in the acid (cyclopropyl) ring.

[b] Females were dosed with bifenthrin labelled with ^{14}C in the alcohol (phenyl) ring.

[c] Values in parentheses are reported as standard deviations.

was complete within 48 h of dosing. A summary of the per cent distribution of bifenthrin following various dosing regimes is given in Table 1.

For the single and multiple low doses, the radiolabel residue in most tissues was <0.1 ppm bifenthrin equivalents, except for fat, pancreas, skin, liver and lungs, which contained higher residues. Mean radioactivity recovered in various tissues was less than 5% of the administered dose after 7 days. The results of this study suggest that orally administered bifenthrin was quickly eliminated from the body (within 48 h after dosing), and no significant differences in elimination or tissue retention were observed following single low and high doses or repeated dosing at the low dose for 14 days (Selim, 1987).

Samples of urine and faeces from the Selim (1987) study were further subjected to metabolic identification. Faecal samples (0–48 h) were pooled from the single low dose or single high dose studies, and 0–72 h faecal samples were pooled from the repeated-dose study. Analysis of rat faeces was done by solvent extraction (equal volumes of acetone and methanol) followed by solvent partitioning (equal volumes of acetonitrile and hexane). Rat urine was partitioned with methylene chloride. The aqueous layer was subjected to acid hydrolysis and repartitioned with methylene chloride and ethyl acetate. After suitable preparation/separation steps, the individual metabolites were identified using HPLC, TLC, gas chromatography–mass spectrometry (GC-MS) or nuclear magnetic resonance (NMR). Faecal extraction of the alcohol- and acid-labelled bifenthrin showed that 51.5–57.8% and 55.3–64.4% of the administered dose were recovered in the acetonitrile fraction (free metabolites), 2.2–3.4% and 2.9–7.0% in the hexane fraction and 11.7–13.7% and 10.7–12.7% as bound residues (post-extraction solids) for both labels, respectively. Analysis of these residues showed that parent chemical was the major product (17.2–44.2% of the administered dose). Twelve other metabolites derived from hydrolysis and oxidation of the parent chemical were also detected. The intact hydroxylated metabolites were identified as 4′-hydroxy-hydroxymethyl-bifenthrin, 3′-hydroxy-hydroxymethyl-bifenthrin, hydroxymethyl-bifenthrin, 4′-hydroxy-bifenthrin, 3′-monomethyl-catechol-bifenthrin and 4′-monomethyl-catechol-bifenthrin, along with the hydrolytic and oxidative-hydrolytic products of bifenthrin, including biphenyl alcohol, 4′-hydroxy-biphenyl alcohol, biphenyl aldehyde, biphenyl acid, trifluoropropenyl (TFP) acid and hydroxymethyl-TFP acid. Unknown metabolites (three) ranged between 0.2% and 3.6% of the total administered dose.

Total organosoluble residues of the urine ranged from 10.6% to 17.6% of the administered dose. Analysis of these fractions indicated that parent chemical was a negligible product (0.1%, possibly due to faecal contamination). The urinary metabolites from hydrolysis and hydrolysis–oxidation products of bifenthrin included TFP acid, hydroxymethyl-TFP acid, biphenyl alcohol, 4′-hydroxy-biphenyl

Table 2. Metabolic distribution of orally administered bifenthrin in the urine and faeces of rats

Metabolic product	Metabolic distribution (% of administered dose)					
	4 mg/kg bw (single low dose)		4 mg/kg bw per day (repeated low dose)		35 mg/kg bw (single high dose)	
	Male[a]	Female[b]	Male	Female	Male	Female
4′-Hydroxy-biphenyl alcohol[c]	NA	2.5	NA	3.3	NA	3.3
Monomethyl-catechol-biphenyl alcohol[d]	NA	0.7	NA	1.3	NA	1.6
4′-Hydroxy-biphenyl acid	NA	0.2	NA	0.4	NA	0.2
Biphenyl acid	NA	1.4	NA	2.1	NA	1.7
TFP acid	3.3	NA	4.8	NA	5.9	NA
4′-Hydroxy-biphenyl acid methyl ester	NA	1.1	NA	1.5	NA	1.0
Cis-, *trans*-hydroxymethyl-TFP acid	4.7	NA	8.6	NA	7.9	NA
Biphenyl alcohol	NA	1.7	NA	2.6	NA	2.1
Biphenyl aldehyde	NA	ND	NA	0.6	NA	1.0
4′-Hydroxy-hydroxymethyl-bifenthrin	2.2	3.7	3.1	4.2	1.8	4.4
3′-Hydroxy-hydroxymethyl-bifenthrin	2.1	3.5	3.0	3.9	1.8	4.1
Hydroxymethyl-bifenthrin	0.7	4.1	2.5	3.5	0.7	2.4
4′-Hydroxy-bifenthrin	1.2	7.4	3.2	7.1	1.8	4.9
3′-Monomethyl-catechol-bifenthrin	3.4	4.9	2.2	4.9	2.0	4.9
4′-Monomethyl-catechol-bifenthrin	3.0	4.3	2.0	4.2	1.7	4.3
Bifenthrin from FMC Corporation	44.2	26.4	25.3	17.2	33.2	22.5
Total	64.6	61.9	54.0	56.8	56.1	58.4

Data extracted from ElNaggar & Wu (1986), Table 18

NA, not applicable; ND, not detected

[a] Males were dosed with bifenthrin labelled with ^{14}C in the acid (cyclopropyl) ring.

[b] Females were dosed with bifenthrin labelled with ^{14}C in the alcohol (phenyl) ring.

[c] Includes 3′-hydroxy-biphenyl alcohol.

[d] Includes 3′- and 4′-monomethyl-catechol-biphenyl alcohol.

alcohol, 3′-hydroxy-biphenyl alcohol, 3′-monomethyl-catechol-biphenyl alcohol, 4′-monomethyl-catechol-biphenyl alcohol, biphenyl acid, 4′-hydroxy-biphenyl acid and 4′-hydroxy-biphenyl acid methyl ester. Four unknown metabolites together did not exceed 1% of the dose. Maximum levels of polar, water-soluble degradate amounted to 5.5% of the dose. A summary of the metabolic distribution of orally administered bifenthrin in the faeces and urine is shown in Table 2.

In summary, the extraction of bifenthrin-derived radioactivity from urine and faecal samples was nearly complete. In faeces, the major identified component was the parent compound (17.2–44.2% of the administered dose). A small quantity of the parent compound (<0.1% of the administered dose) was detected in the urine, possibly due to faecal contamination. Identifiable products in faeces and urine included a parent compound and 16 metabolites. The study results suggest that orally administered bifenthrin had undergone extensive hydrolysis and oxidation (ElNaggar & Wu, 1986).

In another study, the absorption, distribution and metabolism of bifenthrin labelled with ^{14}C in the alcohol (phenyl) ring or in the acid (cyclopropyl) ring were studied in Crl:CD (SD) BR rats following single oral gavage dosing. The dosing regime was as follows: the control group received the vehicle (corn oil) only, the second group received a single dose of 5.43 mg/kg bw, the third group received multiple low doses at 4.0 mg/kg bw for 14 days followed by a single radiolabelled gavage dose and a fourth group received a single dose at 35.71 mg/kg bw. Five male and five female rats were used per group.

Table 3. Distribution of radioactivity recovered in male and female rats in 7 days following oral administration of bifenthrin[a]

Dose group (mg/kg bw)	Sex	Distribution of radioactivity (% of administered dose)				
		Urine[b]	Faeces	Tissues	Carcass	Total
5.43	Male	9.37	83.41	0.14	2.65	95.57
	Female	12.13	74.44	0.15	3.71	90.42
35.71	Male	12.43	75.68	0.13	2.38	90.62
	Female[c]	14.46	71.15	0.22	5.01	90.83
4.0 (repeated dose)	Male	11.96	83.50	0.22	3.15	98.82
	Female	14.29	74.01	0.21	5.33	93.84

Data extracted from Cheng (1988), Table 6-8

[a] Males were dosed with bifenthrin labelled with ^{14}C in the acid (cyclopropyl) ring; females were dosed with bifenthrin labelled with ^{14}C in the alcohol (phenyl) ring.

[b] Includes cage rinse, cage wash and cage wipe.

[c] Tissue and carcass data were reported from the initial group, whereas data for faeces and urine were derived from the repeat group.

Males received acid-labelled bifenthrin, and females received alcohol-labelled bifenthrin. Treated animals were kept individually in metal metabolism cages for collection of urine and faeces. Exhaled air and volatiles were not collected based on a preliminary study indicating that <1.0% of the administered dose was recovered in these fractions. Samples of urine and faeces were collected at the following time intervals: 0–8, 8–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 h post-dosing. Treated rats were sacrificed after 7 days, and radioactivity in various tissues and organs was analysed.

The total radioactivity recovery ranged from 90.4% to 98.82% of the administered dose (Table 3). The majority of the radioactivity was found in the faeces (71.2–83.5% of the administered dose) and urine (9.4–14.5% of the administered dose). Less than 0.22% and 5.33% of the administered dose were recovered in tissues and carcasses, respectively, in 7 days after dosing.

The distribution of radioactivity in various tissues and organs after 7 days is shown in Table 4 following oral dosing of rats with bifenthrin. The highest concentrations of radioactivity were observed in fat (1.1–15.6 ppm). The concentration of radioactivity in fat was highest following a single gavage dose of 35.71 mg/kg bw compared with the two other dose groups.

The study author concluded that the rate of elimination was moderate and that the majority of the radioactivity was excreted into urine and faeces within the first 36 h and 72 h for the low dose groups (5.43 mg/kg bw and repeated-dose group) and high dose group, respectively. No sex differences in ^{14}C elimination or distribution were observed for any of the dosing regimes (Cheng, 1988).

Urine and faecal samples from the Cheng (1988) study were further subjected to metabolic identification. Individual homogenized faecal samples were thawed, and 0–48 h samples were combined into one main group for each label independently by sex for both single low dose and multiple low dose. Samples from single high dose for 0–72 h were also combined by sex and label. The urine samples were also pooled in a similar fashion. The pooled samples of urine and faeces were subjected to suitable extraction and separation steps and analysed for the parent compound and metabolite content by TLC, HPLC and liquid scintillation counting.

Faecal metabolites were excreted primarily as non-conjugates, whereas urinary metabolites were eliminated in both conjugated and non-conjugated forms. The metabolite profile appeared to be similar among the three dosing regimes, whereas the excretion rate appeared to be slower in high-dose rats. A summary of the distribution of bifenthrin and its metabolites is shown in Table 5 for faeces and Table 6 for urine.

Table 4. Tissue distribution after 7 days following oral gavage administration of bifenthrin to rats[a]

Tissue/matrix	Tissue distribution (ppm)					
	5.43 mg/kg bw (single oral gavage low dose)		4.0 mg/kg bw (multiple oral gavage doses)		35.71 mg/kg bw (single oral gavage high dose)	
	Male	Female	Male	Female	Male	Female
Blood	0.021	0.006	0.027	0.008	0.106	0.039
Bone	0.019	0.082	0.026	0.086	0.095	0.381
Brain	0.001	0.006	0.002	0.008	ND	0.044
Carcass	0.141	0.208	0.169	0.258	0.897	2.204
Fat	1.118	1.183	1.443	1.267	7.655	15.601
Hair	0.063	0.031	0.052	0.032	1.120	1.038
Heart	0.010	0.022	0.012	0.035	0.094	0.200
Kidney	0.028	0.041	0.035	0.043	0.163	0.400
Liver	0.082	0.091	0.114	0.113	0.510	0.623
Lungs	0.064	0.105	0.044	0.115	0.189	1.411
Muscle	0.007	0.015	0.014	0.025	0.035	0.182
Ovary	NA	0.118	NA	0.056	NA	0.736
Pancreas	0.056	0.124	0.068	0.118	0.267	0.876
Prostate	0.061	NA	0.010	NA	0.184	NA
Seminal vesicles	0.044	NA	0.015	NA	0.330	NA
Skin	0.143	0.184	0.185	0.214	0.726	2.162
Spleen	0.009	0.018	0.020	0.026	0.031	0.143
Testes	0.007	NA	0.011	NA	0.046	NA
Uterus	NA	0.012	NA	0.006	NA	0.207

Data extracted from Cheng (1988), Table 9-11
NA, not applicable
[a] Males were dosed with bifenthrin labelled with ^{14}C in the acid (cyclopropyl) ring; females were dosed with bifenthrin labelled with ^{14}C in the alcohol (phenyl) ring.

Analysis of metabolite fractions indicated that the major faecal metabolites were primarily derived from hydroxylated parent compound, namely: hydroxymethyl-bifenthrin, 4′-hydroxy-bifenthrin, 3′-hydroxy-hydroxymethyl-bifenthrin, 4′-hydroxy-hydroxymethyl-bifenthrin, 3′-monomethyl-catechol-bifenthrin, 4′-monomethyl-catechol-bifenthrin, dimethoxy-bifenthrin and 4′-methoxy-bifenthrin. Hydrolytic products related to monohydroxylated and dihydroxylated intact parent chemical were also detected, which included 4′-hydroxy-biphenyl alcohol, 4′-hydroxy-biphenyl alcohol, dimethoxy-biphenyl acid, dimethoxy-biphenyl alcohol, 4′-methoxy-biphenyl alcohol, biphenyl alcohol, TFP acid, and *cis*- and *trans*-hydroxymethyl-TFP acid.

Analyses of metabolites from urine fractions indicated that no significant intact metabolites were detected. The majority of ^{14}C residues were from hydrolytic or oxidative degradation, as follows: 4′-hydroxy-biphenyl acid, biphenyl acid, 4′-hydroxy-biphenyl alcohol, dimethoxy-biphenyl acid, 4′-methoxy-biphenyl acid, dimethoxy-biphenyl alcohol, biphenyl alcohol, TFP acid, and *cis*- and *trans*-hydroxymethyl-TFP acid.

In summary, when bifenthrin is dosed orally to rats, it is eliminated primarily in faeces as intact parent compound and its hydroxylated metabolites. A small percentage of the administered dose is eliminated through excretion in urine as either hydrolytic or hydrolytic/oxidative metabolites of the parent chemical and/or its intact metabolites. A mild inductive effect was observed, as the products

Table 5. Profile of metabolites in rat faeces[a]

Metabolic product	% of dose					
	5.43 mg/kg bw (single dose)		4.0 mg/kg bw per day (repeated dose)		35.71 or 42.94 mg/kg bw (single dose)	
	Male	Female	Male	Female	Male	Female
Polar[b]	3.25	3.39	5.06	4.46	2.00	2.36
Hydroxymethyl-TFP acid	NA	1.13	NA	1.31	NA	1.12
TFP acid	NA	1.48	NA	1.68	NA	1.26
4′-Hydroxy-biphenyl acid	0.69	NA	1.32	NA	0.66	NA
Dimethoxy-biphenyl acid	0.41	NA	1.35	NA	0.36	NA
4′-Methoxy-biphenyl acid (biphenyl acid)	0.87	NA	1.32	NA	0.84	NA
4′-Hydroxy-biphenyl alcohol	1.45	NA	4.29	NA	1.78	NA
Dimethoxy-biphenyl alcohol	1.22	NA	1.85	NA	1.19	NA
4′-Methoxy-biphenyl alcohol (biphenyl alcohol)	1.79	NA	2.72	NA	1.35	NA
3′- or 4′-Hydroxy-hydroxymethyl bifenthrin (biphenyl aldehyde)	0.77	3.84	3.37	6.50	1.75	4.22
Hydroxymethyl-bifenthrin	0.93	4.13	2.09	6.74	0.97	3.52
4′-Hydroxy-bifenthrin	1.80	3.98	2.85	5.63	1.48	3.26
3′- or 4′-Monomethyl-catechol	2.25	3.81	3.57	6.17	2.20	4.66
Dimethoxy-bifenthrin	1.80	0.93	1.45	0.96	2.09	0.72
4′-Methoxy-bifenthrin	1.24	0.69	0.97	0.45	1.36	0.83
Bifenthrin	39.22	31.17	25.45	21.80	38.25	35.31
Non-polar[c]	9.08	8.90	5.99	5.41	8.53	3.87
Other unknowns[d]	8.32	4.10	12.32	4.93	5.21	3.81
Water soluble	1.20	0.57	0.81	0.56	0.73	0.07
Post-extraction solids	3.94	3.35	3.87	3.43	2.19	3.85
Total	80.24	71.47	80.65	70.03	72.94	68.85

Data extracted from the study report (Wu, 1988), Table 13
NA, not applicable
[a] Males were dosed with bifenthrin labelled with ^{14}C in the acid (cyclopropyl) ring; females were dosed with bifenthrin labelled with ^{14}C in the alcohol (phenyl) ring.
[b] Products eluted prior to hydroxymethyl-TFP acid.
[c] Products eluted after bifenthrin.
[d] Fractions in entire HPLC run not designated to any specified standard.

in faeces were present to a greater degree as hydroxylated intact chemical and less parent compound in the multiple low dose group compared with those of single-dose rats. The rate of excretion was slower in the high dose group (toxic level) compared with the low dose groups.

The study author suggested that bifenthrin metabolism in the rat is similar to that of other pyrethroids, which also metabolize through typical hydrolytic, oxidative and conjugation processes, as summarized in the proposed metabolic pathway in Figure 2 (Wu, 1988).

In a repeated-dose distribution study, female Sprague-Dawley rats were administered a single daily oral gavage dose of bifenthrin (radiochemical purity >99.0%) labelled with ^{14}C in the alcohol (phenyl) ring at 0.5 mg/kg bw per day in corn oil for 70 days. Three treated rats and one control were

Table 6. Profile of metabolites in rat urine[a]

Metabolic product	% of dose					
	5.43 mg/kg bw (single dose)		4.0 mg/kg bw per day (repeated dose)		35.71 or 42.94 mg/kg bw (single dose)	
	Male	Female	Male	Female	Male	Female
4′-Hydroxy-biphenyl acid	1.74	NA	2.15	NA	1.65	NA
Dimethoxy-biphenyl acid	0.30	NA	0.23	NA	0.16	NA
Biphenyl acid	0.95	NA	1.07	NA	0.90	NA
4′-Methoxy-biphenyl acid	0.09	NA	0.12	NA	0.09	NA
4′-Hydroxy-biphenyl alcohol	0.31	NA	0.42	NA	0.35	NA
Dimethoxy-biphenyl alcohol	0.26	NA	0.34	NA	0.28	NA
Biphenyl alcohol	0.06	NA	0.08	NA	0.08	NA
Bifenthrin	0.00	0.00	0.00	0.00	0.01	0.00
TFP acid	NA	1.85	NA	1.63	NA	2.09
cis-Hydroxymethyl TFP acid	NA	0.78	NA	1.48	NA	1.00
trans-Hydroxymethyl TFP acid	NA	0.58	NA	0.78	NA	0.69
Polar origin	1.92	3.23	3.04	5.49	1.77	3.02
Polar unknowns	1.19	0.78	1.75	1.06	1.25	1.14
Non-polar unknowns	0.05	0.36	0.05	0.42	0.00	0.64
Aqueous	0.67	0.36	1.32	0.41	0.72	0.28
Total	7.54	7.94	10.56	11.27	7.26	8.86

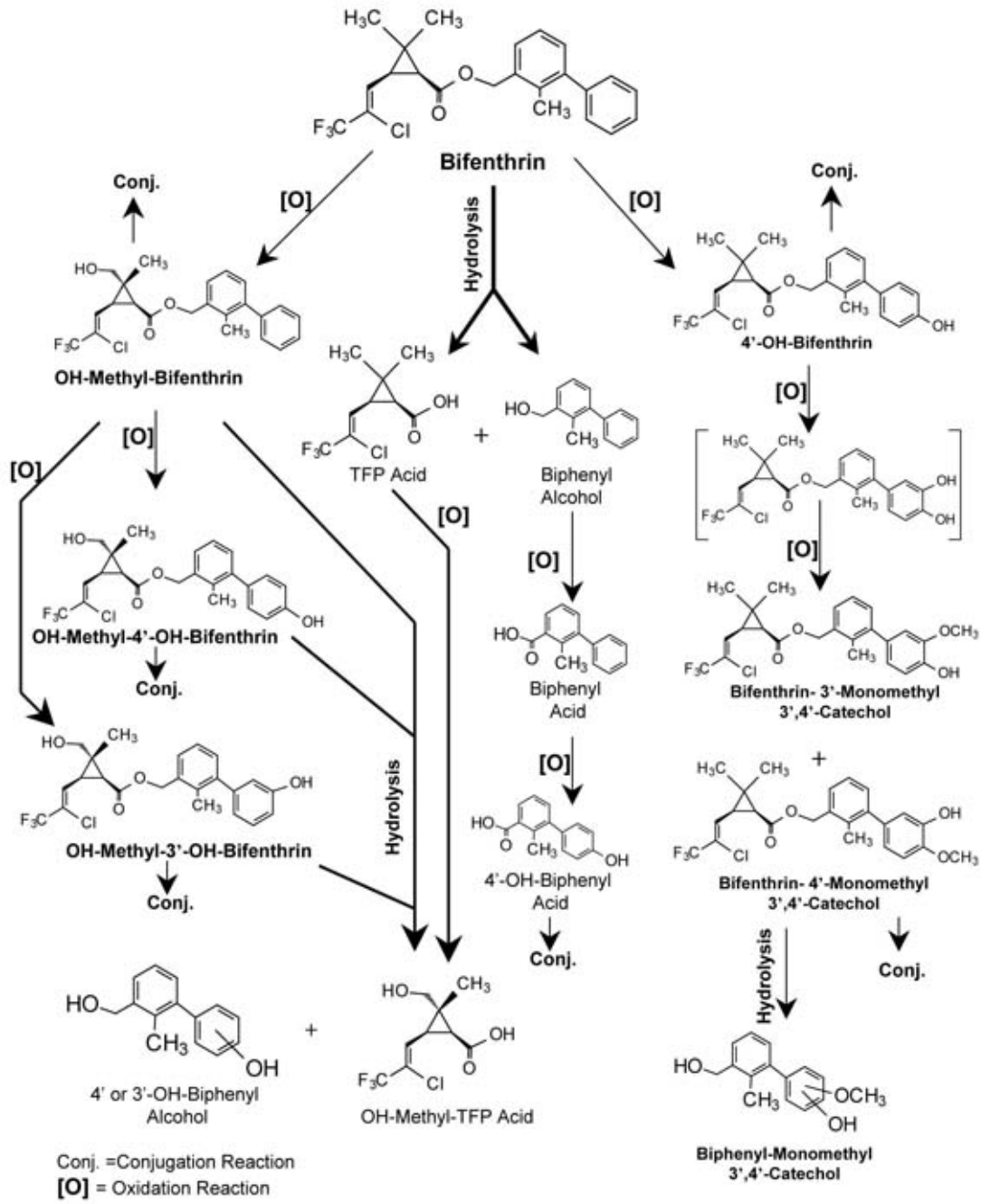
Data extracted from the study report (Wu, 1988), Table 23
NA, not applicable
[a] Males were dosed with bifenthrin labelled with ^{14}C in the acid (cyclopropyl) ring; females were dosed with bifenthrin labelled with ^{14}C in the alcohol (phenyl) ring.

sacrificed periodically during the dosing period, and blood, liver, kidneys, ovaries, fat (sample) and skin were analysed for radioactivity. The sciatic nerve was also removed from animals sacrificed at 56, 63, 70, 73, 99, 113, 127 and 155 days. The nature of radioactivity in the fat was also evaluated using the TLC method. Analysis was extended for an additional 85 days (depuration phase) following cessation of dosing.

At all sacrifice times and in all animals, the highest concentrations of radioactivity were found in fat. Concentrations in the liver, kidney, skin and ovaries were also significantly higher than the corresponding plasma concentrations. Plasma concentrations of radioactivity were similar from day 21 to day 70 (0.04–0.06 µg/ml) and then decreased rapidly to 0.01 µg/ml at 78 days and <0.1 µg/ml at the remaining sacrifice times. The concentrations in the whole blood were very similar to plasma concentrations, indicating some uptake of radioactivity in blood cells but no specific accumulation. Mean concentrations of radioactivity in fat were 0.33 µg/g at 1 day, 0.87 µg/g at 3 days and 2.44 µg/g at 14 days. Concentrations in fat then increased slowly, with mean concentrations of radioactivity between 4.47 and 9.62 µg/g during the remaining dosing period. After the final dose, mean concentrations of radioactivity in fat declined with an approximate half-life of 51 days to 8.79 µg/g, 5.44 µg/g, 4.53 µg/g and 2.74 µg/g at 78, 92, 113 and 155 days, respectively.

Average peak concentrations of bifenthrin radioactivity in fat, skin, liver, kidney, ovaries, sciatic nerve, whole blood and plasma amounted to 9.62 µg/g, 1.74 µg/g, 0.40 µg/g, 0.32 µg/g, 1.69 µg/g, 3.25 µg/g, 0.06 µg/g and 0.06 µg/g, respectively. Estimated half-lives of 51 days (fat), 50 days (skin), 19 days (liver), 28 days (kidney) and 40 days (ovaries and sciatic nerve) were derived from ^{14}C depuration.

Figure 2. Proposed metabolic pathway for bifenthrin in the rat



Analysis of fat samples by hexane extraction, acetonitrile extracts and TLC determined that the presence of unchanged bifenthrin accounted for 65% of the radioactivity in the 1- and 14-day samples and 72–85% in the 7- to 155-day samples. Another component in fat accounted for 5.3% of the total radioactivity on day 1 and 19.5% of the total radioactivity on day 155. The remaining radioactivity in the fat was associated with two polar components (Hawkins, Elsom & Jackson, 1986).

In a plasma kinetics determination study, male Sprague-Dawley rats (Crl:CD (SD) BR) were administered bifenthrin (radiochemical purity 98.0%) labelled with ^{14}C in the alcohol (phenyl) ring as a single oral gavage dose at 4 or 35 mg/kg bw in corn oil. Blood was collected from five treated rats from

each dose group at 1, 2, 3, 4, 6, 8, 10, 12, 48 and 72 h post-dosing. A group of five animals was sacrificed by heart puncture at 2, 4, 10 and 24 h after dosing for the low dose group and at 3, 6, 10 and 24 h after dosing for the high dose group. These time intervals for sacrifice were based on the results of the preliminary study indicating that the peak blood levels were reached between 4 and 8 h after dosing for both the low and the high dose groups. The results of the main study indicated that the blood level of radioactivity for both the low and high dose groups followed a similar pattern. The radioactivity was slowly absorbed, with a time to peak plasma level of 4 h for the low dose and 6 h for the high dose. The decline of radioactivity was slow, with detectable radioactivity at 24 and 48 h after dosing (Selim, 1986).

The plasma samples from the Selim (1986) study were further analysed to identify the nature of the radioactivity. Pooled plasma from five rats from each of five sampling intervals was deproteinized and extracted with acetone, and the whole plasma was analysed by HPLC and liquid scintillation counting. HPLC analysis of the acetone organosoluble residues at the two peak intervals indicated the presence of parent compound (25–38% of the total radiolabel). Biphenyl alcohol (29–33%) and biphenyl acid (21–34%) comprised a majority of the remaining plasma radiocarbon residues. Parent chemical and its metabolites declined in plasma over time, with a corresponding increase in protein-bound residues of the total radiolabel (unextractable residues).

In summary, the results of this study suggest that the disposition of radiocarbon-labelled bifenthrin in rat plasma appears to be primarily hydrolysis of the ester group and oxidation of the resulting alcohol and acids (Tullman, 1986).

Bile duct–cannulated Sprague-Dawley rats were dosed with bifenthrin (radiochemical purity 98.0%) labelled with ^{14}C in the alcohol (phenyl) ring administered as a single gavage dose of 2.7 mg/kg bw to four females and 5.2 mg/kg bw to four males. Corn oil was used as a vehicle. Samples of urine, faeces and bile were collected at 0–12, 12–24, 24–36, 36–48 and 48–72 h. Treated animals were sacrificed at 72 h after dosing, and various organs and tissues were analysed for radioactivity. Faeces, gastrointestinal tract contents and bile samples were further subjected to metabolite identification.

In female rats dosed at 2.7 mg/kg bw, mean excretion of radioactivity was 30.0%, 15.0% and 48.7% of the administered dose in the bile, urine and faeces, respectively, 72 h post-dosing. Approximately 4.8% of the dose in female rats was recovered in the gastrointestinal tract, skin and liver. In male rats dosed at 5.2 mg/kg bw, mean excretion of radioactivity was 18.6%, 10.7% and 24.9% of the administered dose in the bile, urine and faeces, respectively, 72 h post-dosing. Approximately 6.3% of the dose in male rats was recovered in the gastrointestinal tract, skin and liver. The chromatographic profile of the faeces indicated that the majority of the radioactivity was the parent compound, averaging 89.5% and 92.3% of the extractable radiocarbon in female and male rats, respectively. Hydrolytic products resulting from gut microflora in faeces were minor, averaging 10.5% and 7.7% for female and male rats, respectively. The major product in the gastrointestinal tract contents of all samples was unchanged bifenthrin, averaging 84.3% and 91.9% of the extractable radiocarbon for female and male rats, respectively. Hydrolytic degradates and metabolites detected in the gastrointestinal tract contents were minor but slightly higher than in faeces, averaging 15.7% and 8.1% for female and male rats, respectively. Analysis of bile indicated that less than 1% of the radiocarbon was attributable to parent compound, with the remainder composed of polar and conjugated metabolites. The metabolic products in the bile were derived from hydroxylated parent compound, namely: hydroxymethyl-bifenthrin, 4′-hydroxy-bifenthrin, dihydroxy-bifenthrin, bifenthrin-guaiacol (3′- or 4′-monomethyl catechol-bifenthrin) and other hydrolytic products, which included biphenyl alcohol and biphenyl acid. The overall profile of bile metabolites (i.e. aglycones) corresponded closely to the metabolite profile in faeces from the previously described rat metabolism studies.

Total absorption of bifenthrin using the sum of average biliary and urinary excretion and tissue concentrations yielded a value of 49.8% of the administered dose for females and 35.6% of the

dose for males. The results of this bioavailability study suggest that the faecal metabolites and other non-bifenthrin-related radioactivity in the faeces identified in the previously described rat metabolism studies were due to enterohepatic absorption of bifenthrin, biotransformation in the liver and subsequent biliary excretion. In previous studies, virtually no differences in absorption were noted between male and female rats. In this study, differences arose due to differing dosing regime. Male rats were given doses of bifenthrin approximating the low dose in the previous study, but this proved too stressful for the surgically modified rats. The females, studied after the conclusion of the male rat study, were dosed at a lower rate to increase the survivability and health of the animals; consequently, they absorbed a higher percentage of bifenthrin dose. In this regard, it may also be noted that despite the obvious stress on the male rats (evidenced by low biliary excretion and decreased defecation), the ratio of radioactivity distributed between bile and urine as a percentage of the dose (1.7:1) was still very similar to that observed for the females (2:1). This indicates that although absorption may have been impaired, the partitioning of absorbed material between biliary and urinary excretory routes was nevertheless similar to that observed in non-cannulated rats (ElNaggar & Tullman, 1991).

(b) Dermal application

In a percutaneous absorption study, three groups (24 per group) of male rats (Crl:Crn(SD)BR) received single doses of aqueous suspensions of bifenthrin labelled with ^{14}C on the alcohol ring on a previously clipped area of the skin on the back. The rats each received an average of 49.2, 514 or 5252 µg of the test material. Four rats were sacrificed for each dose at 0.5, 1, 2, 4, 10 or 24 h after dermal application. The disposition of the administered ^{14}C was followed. The radioactivity at the application site was extracted and analysed.

The amounts of bifenthrin equivalents eliminated in the urine and faeces of individual rats, even after 24 h of exposure to the test material, were less than 1% of the amount applied to the skin. At the lowest dose level, measurable levels of the test compound did not appear in the excreta until after 10 h of exposure. At the highest dose level, levels of test compound equivalents appeared after 24 h, but the total amount in excreta after 24 h was only 0.2% of the dose. Small quantities of bifenthrin-derived radioactivity were detected in the blood and carcasses. The amount absorbed was defined as the sum of the ^{14}C present in the excreta, the carcass and the skin at and adjacent to the application site (following removal of residual test material with several water washes). There appears to be fairly rapid absorption through the skin during the first half hour after application, and the amounts of absorbed compound did not increase with time. There was also a direct correlation between the concentration applied and the amount absorbed. At 24 h, approximately 70.8%, 44.8% and 53.4% of the dermally applied doses of 49.2, 514 and 5252 µg, respectively, were absorbed (Craine, 1986).

In a second percutaneous absorption study, male Sprague-Dawley rats were dermally dosed with the aqueous emulsion CAPTURE 2EC containing 36.2 µg of bifenthrin (107 kBq, 200 µl). Four rats were killed at 0, 4, 10 and 24 h post-dose. The total amount of ^{14}C radioactivity removed by a skin wash procedure and the total amount of ^{14}C radioactivity remaining on washed, dosed skin were determined. Samples of blood, urine, faeces and residual carcass were collected and analysed for ^{14}C content. The radioactivity recovered in the skin wash was 96.83%, 84.75%, 76.86% and 72.88% of the total administered dose at 0, 4, 10 and 24 h after dermal application, respectively. The radioactivity recovered in the skin application site after wash was 4.04%, 12.00%, 16.55% and 19.44% at 0, 4, 10 and 24 h after dermal application. The sums of the applied dose recovered in blood, plasma and carcass were 0.09%, 0.87%, 0.85% and 1.67% at 0, 4, 10 and 24 h post-application. The sums of the applied dose recovered in urine and faeces were 0.14%, 0.43% and 3.23% at 4, 10 and 24 h post-application. Based on the results of this study, it can be concluded that the dermal absorption (sum of the radioactivity in excreta, blood, plasma and carcass) of aqueous emulsion CAPTURE 2EC containing 36.2 µg of bifenthrin was 1.01%, 1.30% and 4.90% at 4, 10 and 24 h after dermal application (Braun, 1990).

(c) In vitro

An in vitro comparative metabolism study was conducted to evaluate species differences in metabolism of radiolabelled bifenthrin between male and female Swiss-Webster mice and male Sprague-Dawley rats. Bifenthrin labelled with ^{14}C in the cyclopropyl ring (acid-labelled) or in the phenyl ring (alcohol-labelled) was incubated for up to 60 min with liver microsomal metabolizing S9 mix obtained from male and female Swiss-Webster mice or male Sprague-Dawley rats. The reactions were quenched with methanol, the incubates were centrifuged to remove protein and the supernatant was analysed by HPLC. In order to accentuate any differences in metabolism between the incubates, 60 min supernatants were adjusted to pH 10 and extracted with hexane to remove the majority of unmetabolized bifenthrin. Analysis of the 60 min incubates revealed that more than 60% of the radioactivity was present as the unmetabolized bifenthrin. Those metabolites that were observed indicated that bifenthrin had undergone hydroxylation and scission reactions with each liver S9 mix at 60 min. Extractions of the supernatants revealed the presence of up to nine metabolites of bifenthrin, most of which remained unidentified. Comparison of chromatograms from each of the S9 types indicated that one metabolite was found uniquely in male mouse and another only in female mouse.

When incubated with liver S9 mixes, bifenthrin was metabolized by scission and hydroxylation. The extent of metabolism with each liver was in the order male mouse > female mouse > rat. However, the overall rate of in vitro metabolism was low (Kennelly, 1989).

2. Toxicological studies

2.1 Acute toxicity

The acute toxicity of bifenthrin is summarized in Table 7.

(a) Oral administration

Mice

Groups of male and female young adult Swiss-Webster mice (10 of each sex per dose) were given bifenthrin (purity 91.4%) as a single dose at 0, 25, 35, 42 or 50 mg/kg bw by gavage in corn oil. Treated mice were subjected to gross necropsy after 14 days. Animals were observed for mortality and clinical signs of toxicity at 0.5, 1, 2, 3, 4 and 6 h on the day of dosing and twice daily thereafter for 13 days. Body weights were recorded on days 0, 7 and 14 of the study. Clinical signs commonly observed during the study included clonic convulsions, tremors and oral discharge. The onset of these signs began approximately 2 h after dosing and continued to be observed until day 1 of the study, at which time all surviving mice had returned to normal. All survivors gained weight by the end of the study. All deaths occurred within 24 h of dosing. There were no significant treatment-related effects noted at necropsy. The oral median lethal dose (LD_{50}) for bifenthrin in mice was 43.5 (36.2–50.7) mg/kg bw and 42.5 (37.1–47.9) mg/kg bw for males and females, respectively (Rand, 1983a).

Rats

Groups of male and female young adult Sprague-Dawley (Tac:N(SD)fBR) rats (10 of each sex per dose) were given bifenthrin (purity 92.0%) as a single dose at 0, 34, 40, 44, 48, 55 or 67 mg/kg bw by gavage in corn oil. Animals were observed for mortality and clinical signs at 0.5, 1, 2, 3, 4 and 6 h after administration and twice daily thereafter for 13 days. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals. No treatment-related effects on body weight were observed. The clinical signs generally observed at all dose levels included clonic convulsions, tremors and chromorhinorrhoea. The onset of these signs began approximately 3 h after dosing and continued to be observed throughout the day. In addition, animals

Table 7. Acute toxicity of bifenthrin

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Reference
Mouse	Swiss-Webster	M, F	Oral	M: 43.5 (36.2–50.7) F: 42.5 (37.1–47.9)		Rand (1983a)
Rat	Sprague-Dawley (Tac:N(SD)fBR)	M, F	Oral	M: 55.5 (49.7–61.3) F: 53.4 (46.8–60.0)		Norvell (1982)
Rat	Sprague-Dawley (Tac:N(SD)fBR)	M, F	Oral	M: 70.1 (44.5–95.6) F: 53.8 (44.2–63.5)		Freeman (1983)
Rat	Sprague-Dawley CD	M, F	Oral	M: 168.4 (111.4–225.4) F: 210.4 (129.3–291.6)		Watt (1997)
Rat	Sprague-Dawley (Tac:N(SD)fBR)	M, F	Intraperitoneal	M: 770.9 (364.5–1177.4) F: 822.4 (432.8–1212.1)		Kedderis (1985)
Rabbit	New Zealand White	M, F	Dermal	>2000		DeProspo (1983a)
Rat	Sprague-Dawley	M, F	Dermal	>2000		Kedderis (1985)
Rat	Crl:CD(SD)IGS BR	M, F	Inhalation (4 h, nose only)		F: 0.8 (0.5–1.5) M: 1.1 (0.94–1.29)	Kiplinger (2003)
Rabbit	New Zealand White	M, F	Dermal irritation	Non-irritating		DeProspo (1983b)
Rabbit	New Zealand White	M, F	Ocular irritation	Non-irritating		DeProspo (1983c)
Guinea-pig	Dunkin-Hartley	M	Dermal sensitization	Not sensitizing		DeProspo (1983d)
Guinea-pig	Ibm: GOHI; SPF	F	Dermal sensitization	Skin sensitizer		Arcelin (2003)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male

at all dose levels appeared to have abdominal pain following dosing. By day 3, all surviving rats had returned to normal. All deaths occurred within 24 h after dosing. There were no internal gross lesions noted in any animal dying after treatment. Gross signs observed in rats dying after dosing included chromorhinorrhoea, chromodacryorrhoea and abdominogenital staining. The external signs correlate with the clinical observations prior to death. The oral LD_{50} of bifenthrin in rats was 55.5 (49.7–61.3) mg/kg bw and 53.4 (46.8–60.0) mg/kg bw for males and females, respectively (Norvell, 1982).

In a second study, groups of male and female young adult Sprague-Dawley (Tac:N(SD)fBR) rats (10 of each sex per dose) were given bifenthrin (purity 91.4%) as a single dose at 0, 20, 40, 60, 80, 90 or 100 mg/kg bw by gavage in corn oil. Animals were observed for mortality and clinical signs at 0.5, 1, 2, 3, 4 and 6 h after administration and twice daily thereafter for 13 days. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals. No treatment-related effects on body weight were observed. Clinical signs commonly observed during this study included clonic convulsions, tremors, ataxia, loss of muscle control, decreased locomotion, chromorhinorrhoea, chromodacryorrhoea and oral discharge. These signs started approximately 3 h after dosing and continued until day 5. At that time, the surviving animals returned to normal. At necropsy of rats that died during the study, blood in the intestines of three animals and red intestinal lining in one animal were observed. The necropsy of the rats that survived until study termination appeared normal.

The oral LD_{50} of bifenthrin was 70.1 (44.5–95.6) mg/kg bw and 53.8 (44.2–63.5) mg/kg bw for males and females, respectively (Freeman, 1983).

In a third study, groups of male and female young adult Sprague-Dawley CD rats (five of each sex per dose) were given bifenthrin (purity 93.7%) as a single dose at 0, 75, 100, 150, 200 or 300 mg/kg bw by gavage, undiluted. Animals were observed for mortality and clinical signs at 0.5, 1, 2, 3, 4 and 6 h after administration and twice daily thereafter for 14 days. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals. No treatment-related effects on body weight were observed. The most significant clinical signs observed during the study were tremors, vocalization, clonic convulsions, twitching, abdominal gripping and hypersensitivity to touch. Other clinical signs noted included abdominal staining, oral discharge, chromorhinorrhoea, chromodacryorrhoea, diarrhoea and broken tooth. All signs of toxicity ended by day 3 of the study. There were no gross internal lesions noted in any animal during necropsy. The oral LD_{50} of bifenthrin in rats was 168.4 (111.4–225.4) mg/kg bw and 210.4 (129.3–291.6) mg/kg bw for males and females, respectively (Watt, 1997).

(b) Intraperitoneal administration

Groups of male and female young adult Sprague-Dawley (Tac:N(SD)fBR) rats (10 of each sex per dose) were given bifenthrin (purity 88.35%) as a single intraperitoneal dose at 0, 100, 250, 500, 900 or 1500 mg/kg bw in corn oil. Animals were observed for mortality and clinical signs at 0.5, 1, 2, 3, 4 and 6 h post-dosing and twice daily thereafter. Body weights were recorded on day 0 and weekly thereafter. A gross necropsy was performed on all animals. Clinical signs such as tremors, clonic convulsions, chromorhinorrhoea, abdominogenital staining and hypersensitivity to touch were observed 4–6 days post-dosing and continued to be observed for up to 4–5 weeks (500 mg/kg bw) or 6–8 weeks (900 mg/kg bw). Minimal or no clinical signs were observed at 100 or 250 mg/kg bw. Deaths occurred in some animals after dosing but were delayed (1–2 weeks) in most animals. Most animals had white nodules throughout the liver, spleen, diaphragm, stomach or mesentery. These lesions were histologically diagnosed as focal foreign body granulomata, apparently induced by precipitation of the test substance following injection. The intraperitoneal LD_{50} of bifenthrin in rats was 770.9 (364.6–1177.4) mg/kg bw and 822.4 (432.8–1212.1) mg/kg bw for males and females, respectively (Kedderis, 1986).

(c) Dermal application

Rats

Five male and five female young adult Sprague-Dawley rats were exposed dermally to bifenthrin (purity 88.35%) at 2000 mg/kg bw applied undiluted to a Hilltop chamber, then allowed to set for approximately 15 min. The Hilltop chamber was then placed over the intact test site and secured by latex tape. The test substance was maintained in contact with the skin for 24 h. The test site was then washed with a clean gauze pad moistened with acetone, followed by a wipe with tap water. The rats were observed for 14 days. Animals were observed for mortality and clinical signs at 0.5, 1, 2, 3, 4 and 6 h after administration and twice daily thereafter for 13 days. Body weights were recorded on days 0, 7 and 14. A description of the local irritation was recorded on days 1, 3, 7 and 14. A gross necropsy was performed on all animals. There were no deaths. All rabbits lost weight by day 14 of the study. No irritation was observed. Male rats exhibited staggered gait on days 2 and 3. Female rats exhibited staggered gait, decreased locomotion and abdominogenital staining between study days 2 and 4. At necropsy, no gross lesions were observed in any animal. The dermal LD_{50} of bifenthrin in rats was >2000 mg/kg bw for males and females (Kedderis, 1985).

Rabbits

Five male and five female young adult New Zealand White rabbits were exposed dermally to bifenthrin (purity 88.35%) at 2000 mg/kg bw applied to approximately 10% of the (shaved) intact

body surface area. The test substance was maintained in contact with the skin for 24 h using an occlusive wrap. The rabbits were observed for 14 days. Animals were observed for mortality and clinical signs at 0.5, 1, 2, 3, 4 and 6 h after administration and twice daily thereafter for 14 days. Body weights were recorded on days 0, 7 and 14. A description of the local irritation was recorded on days 1, 3, 7 and 14. A gross necropsy was performed on all animals. There were no deaths. All rabbits lost weight by day 14 of the study. All rabbits remained healthy throughout the study. The only signs of irritation observed were erythema in all rabbits 24 h after application and desquamation in four rabbits on day 14 of the study. Postmortem examination revealed pitted kidneys in one rabbit. The dermal LD_{50} of bifenthrin in rabbits was >2000 mg/kg bw for males and females (DeProspo, 1983a).

(d) Exposure by inhalation

Rats

Groups of five male and five female young adult Crl:CD(SD)IGS BR rats were exposed nose only to bifenthrin (purity 94.8%) for 4 h at mean measured (gravimetric method) particulate concentrations of 0.56, 0.99 or 2.33 mg/l. Rats were observed for 14 days. Atmospheres generated had mean aerodynamic particle sizes of 2.0, 2.3 and 2.1 μm. Mortality occurred at 0.56 mg/l (two females), 0.99 mg/l (one male and two females) and 2.33 mg/l (five males and five females). All deaths occurred within 1 day of exposure. At 2.33 mg/l, varying degrees of central nervous system effects, ranging from tremors to convulsions, were noted in the surviving animals immediately after exposure. Three males in this group were euthanized. At 0.99 mg/l, abnormal gait, convulsions, hypothermia, laboured respiration and rales were noted immediately following exposure. There were no other toxicologically relevant clinical findings immediately following exposure. Abnormal gait and tremors were noted in the 0.56 mg/l group immediately following exposure. During the 14-day post-exposure, abnormal gait, convulsions, decreased defecation/urination, increased respiration rate, tremors, unkempt appearance and red/yellow staining on various body parts were noted in the 0.56 mg/l and 0.99 mg/l dose groups. No treatment-related effects on body weight were observed. No effects were noted in animals during the necropsy. The median lethal concentration (LC_{50}) of bifenthrin at 4 h in rats was calculated to be 0.8 mg/l (0.5–1.5 mg/l) for females and 1.1 mg/l (0.94–1.29 mg/l) for males (Kiplinger, 2003).

(e) Dermal irritation

In a study of primary dermal irritation, three young adult male and female New Zealand White rabbits were dermally exposed to 0.5 ml of bifenthrin (purity 88.35%). Eight test sites (intact and abraded) were covered with gauze patches (5 cm × 5 cm) under which 0.5 ml of bifenthrin technical was applied per test site. The trunk of the animal was then wrapped with an elastic gauze bandage. The test material was in contact with the skin for 4 h, after which the animals were unwrapped. Dermal irritation was scored according to the Draize method after 30 min and then daily for 3 days. Clinical signs were recorded daily. No irritation was observed on any rabbits following application of bifenthrin on intact or abraded sites. Under the conditions of this study, it is concluded that bifenthrin is non-irritating to the skin of rabbits (DeProspo, 1983b).

(f) Ocular irritation

In a study of primary eye irritation, 0.1 ml of bifenthrin (purity 88.35%) was instilled into the conjunctival sac of one eye of each of three male and six female young adult female New Zealand White rabbits. The eyes of six of the rabbits remained unwashed, and the eyes of three of the rabbits were gently washed with 100 ml of tap water approximately 20–30 s after treatment. Irritation was scored by the method of Draize at 1 h and 1, 2 and 3 days after exposure. The primary irritation scores for the unwashed eyes were 6.0, 1.0, 0 and 0 at 1, 24, 48 and 78 h post-instillation. The primary irrita-

tion scores for the washed eyes were 8.0, 0, 0 and 0 at 1, 24, 48 and 78 h post-instillation. One hour after dosing, all of the eyes had severe discharge (grade 3), and all of the washed eyes had slight conjunctival redness. At the 24 h reading, two of the unwashed eyes had slight conjunctival redness, one of which also had slight discharge. Within 48 h after instillation, all eyes had returned to normal, and the test was terminated following the 72 h scoring. Under the conditions of this study, it is concluded that bifenthrin is non-irritating to the eyes (washed and unwashed) of rabbits (DeProspo, 1983c).

(g) Dermal sensitization

In a study of dermal sensitization with bifenthrin (purity 88.35%), 10 young male Dunkin-Hartley guinea-pigs were tested using the closed patch technique (Buehler method). One half millilitre of bifenthrin was applied to each of 10 Hilltop chambers. The chambers were applied to the test sites on the left shoulder and secured by a bandage. After 6 h, the bandage and chambers were removed, and the test sites were cleaned. The guinea-pigs were dosed in this manner 3 times a week for a total of 10 treatments. Fourteen days after the tenth treatment, the animals were challenged with the test material at a virgin skin site. Observations for skin reactions (Draize method) were recorded at 24 h after each application. Body weights were recorded at study initiation and termination. 1-Chloro-2,4-dinitrobenzene (0.15% weight by volume in ethanol) was used as the positive control. All animals gained weight by the end of the study. The guinea-pigs that received the test material had tremors within 48 h after the first application. Sporadic incidences of tremors continued to be observed in many of the animals during the dosing period. No irritation was observed in any test animals after induction or challenge application. All of the positive control animals had moderate to severe erythema (nine of which also had necrosis) at challenge. Based on these results, it is concluded that bifenthrin is not a skin sensitizer in guinea-pigs as determined by the closed patch technique (DeProspo, 1983d).

In another study of dermal sensitization with bifenthrin (purity 94.8%), 10 young female SPF guinea-pigs were tested using the maximization method of Magnusson & Kligman. For the main study, 10 female guinea-pigs were assigned to a control group, and 10 to the treatment group. In this study, the test concentrations chosen were 5% for intradermal induction, 5% for topical induction and 3% for the challenge. Polyethylene glycol 300 was used as the vehicle. Skin reactions at the challenge sites were observed at 24 h and 48 h after removal of the patch. One animal was found dead on test day 18. At necropsy, no macroscopic findings were reported. The cause of death was not attributed to the treatment. One animal of the test group was noted with slight tremor on test day 10. Body weights were normal except for the animal that was found dead. Discrete/patchy erythema was observed in eight out of nine animals at the 24 and 48 h readings in the treated animals. No skin reactions were noted in the controls. Based on the results of this study, bifenthrin is a potential sensitizer in guinea-pigs (Arcelin, 2003).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 28-day dose range–finding study of toxicity, groups of 10 male and 10 female Swiss-Webster mice were given diets containing bifenthrin (purity 88.35%) at a dietary concentration of 0, 50, 100, 200 or 300 ppm (equivalent to 0, 7.5, 15, 30 and 45 mg/kg bw per day) for 28 days. The low dose of 50 ppm was changed to 500 ppm after 2 weeks. In a separate study, groups of 10 male and 10 female Swiss-Webster mice were given diets containing bifenthrin (purity 88.35%) at a concentration of 0, 500, 600, 750 or 1000 ppm (equivalent to 0, 75, 90, 112.5 and 150 mg/kg bw per day) for

28 days. Diets were prepared at the initiation of the study and biweekly thereafter. Diets were kept at room temperature. Mice were inspected daily for signs of toxicity and mortality. Body weight and food consumption were measured weekly. At termination, surviving mice were subjected to a gross necropsy, and organ weights were obtained for the brain, heart, liver, gonads, adrenals and kidneys.

In this study (doses up to 300 ppm), there were no significant treatment-related effects. Slightly decreased absolute brain weights were observed at the 200 and 300 ppm dose levels. At doses up to 1000 ppm, all female mice died by day 12, and 7 of 10 males died by day 7. At 750 ppm, five females died by day 6, and at 600 ppm, two females died by day 7. There was no mortality in males in the 600 and 750 ppm dose groups. No mortality was observed in the 500 ppm dose group and below. Prior to death, the most consistent clinical signs of intoxication were clonic convulsions and tremors. Tremors were also consistently observed in the three surviving 1000 ppm males until termination. Food consumption was also significantly lower in these three males compared with controls for the first 3 weeks of the study. However, in the final week, their food consumption was less than that of the controls, but not significantly. In the 600 ppm group, mean body weight was significantly greater in males compared with controls during the last 2 weeks of the study. Body weight gain and brain and liver weights of males were also significantly greater. The only marked aberrant clinical observation noted in males and females of the 500 ppm group was tremors. Under the study conditions utilized, the NOAEL for mice is 300 ppm, equivalent to 45 mg/kg bw per day, based on clinical signs, mortality, body weight changes and organ weight changes seen at 500 ppm, equivalent to 75 mg/kg bw per day, and above (Rand, 1983b).

Rats

In a 28-day study of toxicity, groups of 10 male and 10 female Sprague-Dawley rats were given diets containing bifenthrin (purity 91.4%) at a concentration of 0, 50, 100, 200, 300 or 400 ppm (equivalent to 0, 5, 10, 20, 30 or 40 mg/kg bw per day) for 28 days. Diets were prepared at the initiation of the study and at two other times during the study and stored at room temperature. Dietary concentrations were confirmed analytically. Rats were inspected daily for signs of toxicity and mortality. Body weight and food consumption were measured weekly. At termination, surviving rats were subjected to a gross necropsy, and organ weights were obtained for the brain, heart, liver, gonads, adrenals and kidneys.

All rats in the 400 ppm group died by day 15 of the study. In the 300 ppm group, 6 of 10 males died by day 12, and 1 of 10 females died by day 20 of the study. Prior to death, the most consistent clinical signs of intoxication were clonic convulsions and tremors. Mean body weight was significantly depressed for males and females during weeks 1, 2, 3 and 4. Furthermore, the overall gain in weights of males and females throughout the study was significantly less than that of the controls. Adrenal weights were significantly elevated, whereas testicular weights were depressed among males. Organ weights of the females were similar to those of controls. Relative organ to body weight ratios were significantly elevated for the adrenals, brain and kidneys of males and for the brain, kidneys and liver of females. In the 200 ppm group, mean food consumption was significantly depressed during week 1 in females, and mean body weight was depressed in males during week 1. Tremors in males and in females were the most consistent treatment-related observation noted. There were no treatment-related effects observed in rats in the 50 or 100 ppm groups.

Under the conditions of this study, it may be concluded that the NOAEL for bifenthrin in rats is 100 ppm, equivalent to 10 mg/kg bw per day, based on clinical signs seen at the lowest-observed-adverse-effect level (LOAEL) of 200 ppm, equivalent to 20 mg/kg bw per day (Rand, 1983c).

In an oral study of toxicity in rats, bifenthrin (91.4% active ingredient [ai]) was administered for 90 days in the diet to Sprague-Dawley rats (15 of each sex per dose) at dose levels of 0, 12, 50, 100 and 200 ppm (equal to 0, 0.88, 3.8, 7.5 and 15 mg/kg bw per day for males; 0, 1.04, 4.3, 8.5 and

17.2 mg/kg bw per day for females). An additional 10 rats of each sex were administered the diet containing bifenthrin at 0 or 200 ppm for 90 days and then fed a recovery (control) diet for an additional 28 days. Diets were prepared at the initiation of the study and stored at room temperature. Stability, homogeneity and dietary concentrations were confirmed analytically. All rats were examined daily for signs of toxicity and mortality. Body weight and food consumption were measured weekly. At termination, blood was taken for haematological and clinical chemistry analysis from 10 rats of each sex per dose. Ophthalmoscopic examinations were performed on all rats at initiation of the study and during the last week of the study. Animals were then subjected to a gross necropsy, and organ weights were obtained for the brain, gonads, heart, kidneys, liver and adrenals. Histopathological examination of selected tissues was conducted on all animals in the control and the 200 ppm groups. In addition, histopathological examination was performed on the lungs, liver and kidneys of animals in all dose groups.

There was no mortality during the study. There were no treatment-related changes noted in haematological, clinical chemistry, organ weight or gross and microscopic data. In addition, there were no ophthalmological changes observed that were related to dietary levels of bifenthrin. Tremors were noted in 3 out of 15 males and females during the first 2 weeks of the study at 100 ppm and throughout the study at 200 ppm. At 200 ppm, the tremors were reversible within 3 days after discontinuation of dosing. Sporadic changes in body weight and food consumption were noted initially, but these were considered not treatment related, as a dose–response relationship was not apparent and changes were within biological variability. Slightly decreased blood urea nitrogen in the high-dose males and females and higher serum potassium levels in high-dose females were minor and lacked any histopathological correlates.

Under the study conditions utilized, the LOAEL for this 90-day study is 100 ppm, equal to 7.5 mg/kg bw per day for males and 8.5 mg/kg bw per day for females, based on the increased incidence of tremors in both sexes. The NOAEL is 50 ppm, equal to 3.8 mg/kg bw per day for males and 4.3 mg/kg bw per day for females. The study author set the NOAEL at 100 ppm (7.5 mg/kg bw per day for males; 8.5 mg/kg bw per day for females) because the author did not consider tremors seen in 3 out of 15 animals of both sexes at 100 ppm as adverse (Rand, 1984).

Dogs

In a 90-day study of toxicity, groups of four male and four female Beagle dogs were given gelatine capsules containing bifenthrin (purity 88.35%) at a dose of 0, 2.5, 5.0, 10 or 20 mg/kg bw per day for 13 weeks. The dogs were inspected twice per day for mortality, moribundity and clinical signs. Eyes were examined by indirect ophthalmoscopy prior to initiation of dosing and at week 13. Body weight and food consumption were measured weekly. Blood for measurement of haematological and clinical chemistry parameters was collected from all dogs before the test and after 4, 8 and 13 weeks of treatment. At the end of the study, a complete gross postmortem was done. Selected organs were weighed, and a comprehensive range of tissues were preserved and examined microscopically. Stability of the test material was analysed at weeks 5, 9 and 13, and homogeneity was measured during week 7.

There was no mortality during the study. There were no treatment-related changes noted in food consumption, haematology, clinical chemistry, organ weights or gross and microscopic parameters, except for 41.1% and 58.8% reductions in mean absolute ovary weights in the 10 and 20 mg/kg bw per day group females, respectively, relative to controls, presumably because estrus did not occur. In addition, there were no treatment-related ophthalmological changes, although one high-dose male had bilateral epiphora (overflow of tears) at week 13 that may have been treatment related. Tremors were noted in three dogs of each sex at 5.0 mg/kg bw per day and in four dogs of each sex at 10 and 20 mg/kg bw per day. There was a definite increase in the incidence of tremors as the study continued, indicating a cumulative effect. Ataxia was noted in four dogs of each sex at 10 and 20 mg/kg bw per

day and in one female at 5.0 mg/kg bw per day. Languidness occurred primarily at 20 mg/kg bw per day in both sexes, with isolated findings at 5.0 and 10 mg/kg bw per day. All of these symptoms occurred more frequently during the last 3 weeks of the study. Other dose-related clinical signs included blinking, mydriasis, nystagmus, lacrimation and polypnoea (increased rate of respiration) in the two highest dose groups. One high-dose female appeared thin or dehydrated during the final weeks of the study. A non-statistically significant, but possibly treatment-related, reduction in mean body weight gain was noted in females at 20 mg/kg bw per day (0.6 kg) relative to the controls (1.3 kg). None of the females at 10 or 20 mg/kg bw per day showed cyclic activity or signs of estrus, but cyclic activity was observed in two, two and one female at 0, 2.5 and 5.0 mg/kg bw per day, respectively, and four of five showed signs of estrus. A minor decrease in the red blood cell mass was observed, especially in the 10 and 20 mg/kg bw per day dose groups, whereas slight decreases in sodium and potassium levels were noted in some treated groups at selected intervals during the study. The effects were of a subtle nature and were considered to be of little biological importance.

The LOAEL for this 13-week study is 5.0 mg/kg bw per day, based on the increased incidence of tremors in both sexes. The NOAEL is 2.5 mg/kg bw per day (Serota, 1984).

In a 1-year study of oral toxicity, groups of four male and four female Beagle dogs were given gelatine capsules containing bifenthrin (purity 88.35%) at a dose of 0, 0.75, 1.5, 3.0 or 5.0 mg/kg bw per day for 52 weeks. The dogs were inspected twice per day for morbidity or mortality, with clinical signs being checked daily. Body weight and food consumption were recorded weekly through week 13 and every 4 weeks thereafter beginning in week 16. Eyes were examined by indirect ophthalmoscopy at the start of the study and during week 52. Clinical chemistry parameters were measured at week 26 and week 52. Haematology parameters were measured and urinalysis was performed prior to treatment and at weeks 13, 26 and 52. At the end of the study, a complete gross postmortem was done. Selected organs were weighed, and a comprehensive range of tissues were preserved and examined microscopically. Samples were taken at weeks 14, 16, 24, 32, 40 and 52 and sent to the sponsor for stability testing.

No mortality occurred during the study, and there were no treatment-related effects on food consumption, organ weights, ophthalmology, urinalysis or gross and microscopic pathology. Mean body weight gains in the 5.0 mg/kg bw per day males were 42% lower than those of controls during weeks 0–24 and 56% lower than those of controls during weeks 0–52. The decreased body weight gain in the high dose group males, generally observed following 11 weeks of treatment, is suspected to be treatment related, as the timing was consistent with the timing of delayed response of tremors observed in this group. Tremors were noted in all males and females at 5.0 mg/kg bw per day during weeks 15–29 and in one of four males and two of four females at 3.0 mg/kg bw per day during weeks 16–23, with the effects somewhat more pronounced in the males. The lack of tremors after 29 weeks suggests that dogs may have developed a tolerance to treatment. In seven of the dogs (two from the 3.0 mg/kg bw per day group and five from the 5.0 mg/kg bw per day group), tremors were noted prior to the daily dose, indicating a persistent effect from the previous day's dose. There was a slight, non-significant decrease in red blood cells, haematocrit and haemoglobin at 26 and 52 weeks in the 5.0 mg/kg bw per day males and females. A significant increase in platelets was noted at 52 weeks in the 5.0 mg/kg bw per day males. Serum sodium levels were significantly increased in males at 3.0 and 5.0 mg/kg bw per day, and serum chloride was increased in males at 5.0 mg/kg bw per day. There was a significant increase in creatinine phosphokinase in the 1.5 and 5.0 mg/kg bw per day females, which was considered to be of questionable biological significance: no clear effect was seen in the 3.0 mg/kg bw per day females, there was considerable variation in individual values, there were a limited number of dogs per group and there was no histological correlate.

The LOAEL for this 52-week study is 3.0 mg/kg bw per day, based on the increased incidence of tremors in both sexes. The NOAEL is 1.5 mg/kg bw per day (Serota, 1985).

(b) Dermal application

Rats

In a repeated-dose dermal toxicity study, groups of 10 male and 10 female Charles River Sprague-Dawley CD rats received dermal applications of undiluted bifenthrin (purity 93.2%) at doses of 0, 25, 50, 100 or 1000 mg/kg bw per day for 6 h/day, 5 days/week, for 3 weeks. The control group animals were wrapped but received no vehicle or other treatment. An area of approximately 10% of the total body surface area on the back of each rat was clipped free of hair prior to study initiation and as necessary thereafter. The appropriate volume of liquefied bifenthrin was applied neat to the skin under a four-ply 5 cm × 5 cm gauze pad using a syringe or microlitre pipette. The gauze patch was held in place with hypoallergenic tape and was wrapped with a self-adhesive bandage. After 6 h, the dressings were removed, and the treated sites were rinsed with acetone, followed by tap water. Animals received treatments for 5 consecutive days per week over a 3-week period. Animals were examined for mortality and signs of toxicity twice daily. Animals were weighed pretest and weekly throughout the study. Individual food consumption was calculated weekly. Ophthalmoscopic examination was performed on all rats before the start of dosing and prior to termination. At termination, animals underwent functional observational battery (FOB) and motor activity testing. At termination of the dosing period, haematological and clinical chemistry determinations were performed, and each animal was examined externally and internally for macroscopic changes. Selected organs were weighed, and a comprehensive range of tissues were preserved and examined microscopically.

There were no treatment-related deaths and no biologically significant treatment-related effects on body weight, body weight gain, food consumption, motor activity, ophthalmoscopic examination or organ weights. Slight haematological and clinical chemistry effects were considered toxicologically insignificant because of small magnitude and the lack of a dose–response relationship. Treatment-related dermal effects included desquamation and eschar on 25 mg/kg bw per day males and females and on 50 mg/kg bw per day females; eschar on 50 mg/kg bw per day males; eschar and erythema on 100 mg/kg bw per day males; and eschar, desquamation and ulceration on 100 mg/kg bw per day females. Eschar, erythema and ulceration were noted in 932 mg/kg bw per day males and females. Hyperplasia and hyperkeratosis were observed at the treatment sites of all groups, including controls, compared with untreated skin. The severity was similar between controls and groups treated with 25, 50 and 100 mg/kg bw per day; however, in 1000 mg/kg bw per day animals, the hyperplasia was more severe than in controls and was sometimes associated with ulceration and secondary dermal inflammation. Dermal effects appeared to be more frequent and more severe in females than in males. Clinical signs suggestive of systemic toxicity occurred in 100 mg/kg bw per day males and females and in 1000 mg/kg bw per day females and included tremors, staggered gait and exaggerated hind limb flexion. Effects were noticeably more prevalent in females than in males. One male treated with 100 mg/kg bw per day exhibited staggered gait, and one female treated with 100 mg/kg bw per day exhibited exaggerated hind limb flexion and staggered gait. No significant clinical signs were noted in 1000 mg/kg bw per day males. Exaggerated hind limb flexion was noted in 4 of 10 high-dose females, staggered gait in 1 of 10 high-dose females and tremors in 9 of 10 high-dose females. Decreases in tail flick latency were noted in females dosed with 50 (34%), 93 (22%) and 1000 (25%) mg/kg bw per day, whereas an increase in tail flick latency was noted in the 1000 mg/kg bw per day males (42%) compared with controls. Based on the trend test, only the 100 and 1000 mg/kg bw per day female results and 1000 mg/kg bw per day male results were statistically significant. Clinical signs attributed to cutaneous paraesthesia from synthetic pyrethroids included vocalization, thrashing in the cage and lying on the back. These effects were noted in one female each at 25, 50 and 100 mg/kg bw per day and in 5 of 10 females at 1000 mg/kg bw per day. Vocalization was observed in one male each at 100 and 1000 mg/kg bw per day.

The systemic NOAEL for bifenthrin in rats is 50 mg/kg bw per day, and the systemic LOAEL is 100 mg/kg bw per day, based on staggered gait and exaggerated hind limb flexion. The dermal

LOAEL is ≤25 mg/kg bw per day (the lowest dose tested) based on slight eschar and desquamation in both sexes. A dermal NOAEL was not identified (Watt & Freeman, 2000).

Rabbits

In a 21-day study of dermal toxicity after repeated doses, groups of six male and six female New Zealand White rabbits received dermal applications of bifenthrin (purity 88.35%) at a dose of 0, 25, 50, 100 or 500 mg/kg bw per day, 6 h/day, for a total of 21 consecutive days. The hair was clipped from the dorsal trunk of each rabbit before the first application, then periodically as required. The test material was applied neat, then wrapped in occlusive gauze bandage covered by a patch of plastic film, and necks were fitted with an everted plastic collar. After the exposure, the gauze and plastic wrapping were removed, and the application site was cleansed free of any residual test material using an acetone-wetted gauze pad and then a water-wetted pad. The rabbits were observed twice per day for signs of toxicity, mortality and morbidity. Food consumption and body weight were recorded weekly. Blood was taken at the end of the study, and the standard test parameters were examined. At the end of the study, all rabbits were examined grossly postmortem. Histopathological examination of liver, kidney and skin from the control and 500 mg/kg bw per day dose groups was performed.

There were no treatment-related differences in body weights, food consumption, urinalysis, haematology, clinical chemistry, or gross and microscopic pathology. One female rabbit in the 500 mg/kg bw per day dose group died on day 19. This death was attributed to ingestion of the test substance, as this animal was found on the day of death without its everted plastic collar. Tremors were noted in three of six males (days 9–20) and two of six females (day 19) at 500 mg/kg bw per day. Tremors were also noted (on day 17) in one of six females at 100 mg/kg bw per day. Loss of muscle coordination was noted in all males (days 2–21) and females (days 2–21) at 500 mg/kg bw per day. This toxic sign appeared as early as 2 days after dosing began and persisted to the end of the study period. Relative liver and kidney weights were significantly elevated in females of the 500 mg/kg bw per day group without any histopathological correlates.

The systemic LOAEL is 500 mg/kg bw per day, based on the loss of muscle coordination and tremors in both sexes. The systemic NOAEL is 100 mg/kg bw per day (DeProspo, 1984a).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity, groups of 50 male and 50 female Swiss-Webster Tac(SW)fBR mice were given diets containing bifenthrin (purity 88.35%) at a concentration of 0, 50, 200, 500 or 600 ppm (equal to 0, 7.6, 29, 74 and 92 mg/kg bw per day for males and 0, 10, 37, 93 and 110 mg/kg bw per day for females) for 87 weeks (males) or 92 weeks (females). The control and treated feeds were sealed in a plastic bag and a closed container and stored at room temperature until distributed to the mice. Stability, homogeneity and dietary concentrations were confirmed analytically. The mice were inspected twice daily for mortality. Changes in clinical condition or behaviour were recorded daily. Body weight and food consumption were measured weekly for the first 13 weeks and then at monthly intervals thereafter. A blood smear was obtained from 10 mice of each sex per dose at 12 and 18 months and also at termination for determining differential blood count data. Clinical chemistry, ophthalmological examination and urinalysis were not performed. All mice that died and those that were sacrificed on schedule were subjected to gross pathological examination. At termination, organ weights were obtained for brain, kidneys, liver and gonads from 10 animals of each sex per group. Histopathological examination of selected tissues was conducted on all animals in the control and high dose groups and also on animals that died or were sacrificed during the study. In addition, histopathological examination was performed on the lungs, liver, sciatic nerve, spinal cord, kidneys, urinary bladder and stomach of animals in the 50, 200 and 500 ppm groups.

Table 8. Non-neoplastic findings in a carcinogenicity study in mice

	Males					Females				
	Dietary concentration (ppm)									
	0	50	200	500	600	0	50	200	500	600
Stomach										
Total evaluated (*N*)	49	50	50	50	49	50	50	50	50	48
Glandular hyperplasia (*N*)	6	8	7	9	8	5	6	5	5	9
% of total	12	16	14	18	16	10	12	10	10	19
Eye										
Total evaluated (*N*)	48	29	25	36	49	49	37	35	29	49
Retinal atrophy (*N*)	14	12	8	11	24	14	12	11	8	23
% of total	29	41	32	31	49*	29	32	31	28	47*
Testes										
Total evaluated (*N*)	49	32	26	38	49	—	—	—	—	—
Bilateral germinal epithelial degeneration (*N*)	4	8	8	8	12	—	—	—	—	—
% of total	8	25*	31*	21	24*	—	—	—	—	—

Data extracted from Geiger (1986), Vol. 1, Table B, p. 28
* $P \leq 0.05$ (Fisher's exact test)

Stability, homogeneity and dietary concentrations were within acceptable ranges. There were no statistically significant differences in survival; however, two females and two males in the high dose group and one female in the 500 ppm group died after 1–2 weeks with compound-related symptoms. Tremors were noted in all males and females at 500 ppm and 600 ppm during the first 3 months of the study. Tremors were also noted in two males and two females at 200 ppm and in one male at 50 ppm. However, tremors in the 50 ppm male occurred late in the study, and this animal died soon after, with fluid detected in the lungs. Tremors in 500 ppm and 600 ppm animals occurred mostly during the first 60 days of the study. Tremors in the 200 ppm males were observed on days 37–39, a period when tremors were quite frequent in the 500 and 600 ppm mice. High-dose males and females also displayed clonic convulsions, jerks and twitching. The mean body weight of males was significantly reduced in the 600 ppm group from weeks 2 to 20 and also in the 500 ppm group on weeks 5, 8, 9, 12 and 13. The mean body weight of the females was significantly reduced in the 500 and 600 ppm groups on weeks 1–6. A treatment-related depression in food consumption was observed for both sexes during the first week of the study only. Differential blood count values were comparable between the 600 ppm dose group and the controls at 12 months, 18 months and study termination. There were no compound-related findings observed at necropsy, and no effects on organ weights were observed. There were slight increases in incidences of glandular hyperplasia of the stomach (not significant by Fisher's exact test) and retinal atrophy (statistically significant by Fisher's exact test) in males and females of the 600 ppm dose group. High-dose (600 ppm) male rats also showed an increased incidence of cortical atrophy of the adrenal gland and bilateral germinal epithelial degeneration of the testes; the latter condition was also significantly elevated in males at 50 ppm and 200 ppm (Table 8).

The incidence of lymphoblastic lymphosarcoma/leukaemia was higher in the high-dose females (22/49 [45%]) than in controls (12/50 [24%]). A pairwise comparison with the controls using Fisher's exact test was statistically significant ($P = 0.24$) at the high dose in females. However, when high-dose female mice with leukaemia were combined with high-dose females with other types of

Table 9. Neoplastic findings in a carcinogenicity study in mice[a]

	Males					Females				
	Dietary concentration (ppm)									
	0	50	200	500	600	0	50	200	500	600
Lymphoblastic leukaemia										
Total evaluated (*N*)	48	50	50	50	49	50	50	50	50	49
Lymphosarcoma/leukaemia (*N*)	—	—	—	—	—	12	14	17	10	22
% of total	—	—	—	—	—	24	28	34	20	44*
Composite lymphosarcoma (*N*)	—	—	—	—	—	7	4	3	6	1
% of total	—	—	—	—	—	14	8	6	12	2
Combined incidence (*N*)	—	—	—	—	—	19	18	20	16	23
% of total	—	—	—	—	—	38	36	40	32	47
Lung										
Total evaluated (*N*)	49	50	50	50	49	50	50	50	50	48
Bronchoalveolar adenocarcinoma (*N*)	21	20	22	17	18	14	25	23	16	22
Bronchoalveolar adenoma (*N*)	4	7	3	1	3	0	1	0	3	1
Combined incidence (*N*)	25	27	25	18	21	14	26	23	19	23
% of total	51	54	50	36	43	28	52*	46*	36	48*
Hepatocellular neoplasms										
Total evaluated (*N*)	49	50	50	50	49	—	—	—	—	—
Adenocarcinoma (*N*)	0	0	1	2	2	—	—	—	—	—
Adenoma (*N*)	2	2	3	2	5	—	—	—	—	—
Combined incidence (*N*)	2	2	4	4	7	—	—	—	—	—
% of total	4	4	8	8	14	—	—	—	—	—
Urinary bladder										
Total evaluated (*N*)	48	50	50	50	49	50	50	50	50	48
Leiomyosarcoma (*N*)	2	6	8	7	14	1	2	4	1	0
% of total	4	12	16	14	29**	—	—	—	—	—

Data extracted from Geiger (1986), Vol. 1, Table A, p. 23
* $P \leq 0.05$; ** $P \leq 0.01$ (Fisher's exact test)

lymphoreticular neoplasms, the overall incidence of lymphoreticular neoplasms was not unlike that experienced by control females (Table 9). A pairwise comparison with the controls using Fisher's exact test indicated increased incidences of bronchoalveolar adenocarcinomas and adenomas in females in the 50, 200 and 600 ppm dose groups. However, time-to-tumour tests for positive trend and heterogeneity indicated that there was no significant trend and that the incidence rates were not significantly different between groups. The hepatocellular tumour incidence rate was elevated in high-dose males (Table 9); however, no predisposing hepatic changes were present in the livers of treated animals. The combined incidence of adenomas and adenocarcinomas in male livers shows that none of the treatment groups were significantly different from the control group as judged by pairwise comparisons with the control using Fisher's exact test. Time-to-tumour tests showed a significant positive trend ($P = 0.022$) in conjunction with a non-significant ($P = 0.354$) test for heterogeneity. Considering other historical control data using CD-1 Swiss-Webster mice in which the incidence of hepatocellular carcinomas ranged from 6% to 28% and the incidence of hepatocellular adenomas

ranged from 0% to 16%, it was concluded that the hepatocellular neoplasms were unlikely to have been treatment induced.

Treated male mice exhibited a dose-related increase in the incidence of leiomyosarcomas in the urinary bladder. Time-to-tumour tests for positive trend and heterogeneity were also significant and support the conclusion that the prevalence and onset of this neoplasm were increased in male mice by administration of bifenthrin in the diet. Female mice did not exhibit this pattern of response.

The LOAEL in this study is 200 ppm, equal to 29 mg/kg bw per day, based on the incidence of tremors in both sexes. The NOAEL in this study is 50 ppm, equal to 7.6 mg/kg bw per day (Geiger, 1986).

The mouse urinary bladder tumours (leiomyosarcomas) from the Geiger (1986) study were further evaluated by transmission electron microscopy (TEM) by Wilborn, Hyde & McConnell (1988). In this evaluation, six small formalin-fixed specimens supposedly of urinary bladders from male mice that were believed to contain tumours were evaluated by the TEM technique. Two urinary bladders were free of tumour cells. They exhibited the three histological layers characteristic of normal bladder: mucosa, muscularis and adventitia. Ultrastructurally, smooth muscle cells of the muscularis in tumour-free bladders and in bladders with tumours appeared to be uninvolved in tumour histogenesis and exhibited the usual features of smooth muscle, including parallel arrays of myofilaments, dense bodies into which myofilaments inserted, surface pinocytotic vesicles and attachment plaques. Most myofilaments were of the actin (thin) type. They were swollen due to the fixative employed, and myosin (thick) myofilaments were difficult to distinguish. A basal lamina rich in collagen microfibrils and sometimes discontinuous focally surrounded individual smooth muscle cells. Nuclei, mitochondria, polyribosomes and segments of rough endoplasmic reticulum were recognizable, but not well preserved by formalin fixation. Tumour cells occupied, and sometimes filled or even distended, the lamina propria. They had unequivocal features of smooth muscle. It is generally agreed that only two features are essential for the diagnosis of smooth muscle tumours—bundles of myofilaments and dense bodies. These and other features are observed in the tumour cells that are characteristic of smooth muscle, and it is evident that the tumour is of smooth muscle origin.

The study authors concluded that evaluation of the formalin-fixed specimens of mouse urinary bladder by TEM has shown that the tumour cells in the lamina propria are derived from smooth muscle (Wilborn, Hyde & McConnell, 1988).

Increased incidences of leiomyosarcomas of the urinary bladder in male mice, combined hepatocellular adenomas and adenocarcinomas, also in male mice, and combined bronchoalveolar adenomas and adenocarcinomas in female mice were observed in the carcinogenicity study by Geiger (1986). These tumours in mice were re-evaluated by an expert panel of three pathologists. This panel of three pathologists found five additional bladder lesions in control mice and concluded that the mouse bladder tumour was not a leiomyosarcoma but rather that the tumour arose in the submucosa, has an unknown pathogenesis, may arise from the vascular mesenchyme, has low malignant potential, occurs predominantly in males and apparently occurs only in mice. The panel considered that the top dose response was equivocal and failed to provide persuasive evidence of a compound-related effect. In a review of liver tumours in male mice, the panel also identified four additional lesions (one each in controls and the 500 ppm dose group, and two in the 50 ppm dose group). Statistical evaluation of adenoma/hyperplasia and carcinoma, both separately and combined, showed no significant difference between control and any treated group on a pairwise comparison and no evidence of a statistically significant trend for adenoma/hyperplasia. While the trend analysis of carcinoma alone showed a significance of $P = 0.024$, the level of significance did not achieve the $P = 0.01$ that would be required for statistical significance of a common tumour in the absence of a

significant pairwise comparison. There was no effect in female mice. The effect in male mice was not considered to be related to the treatment with bifenthrin. The panel reclassified lung adenomas and carcinomas in accordance with widely accepted historical diagnostic criteria. The incidence of adenomas and adenocarcinomas, whether considered separately or in combination, showed no evidence of a significant trend or significant pairwise comparison between controls and the highest dose group. Based upon re-evaluation of the liver and lung pathology, the panel found no evidence of a compound-related effect on the incidence of either benign or malignant liver or lung tumours. The panel found equivocal evidence of bladder tumours in male mice. The panel concluded that the bladder tumour is predominantly benign, is probably vascular in origin and has no relevance to humans (Butler, 1991a).

Additionally asked questions by the USEPA on the histopathological review of the oncogenicity lifetime feeding study in mice were answered by the reviewer (Butler, 1991b) in the addendum, as follows:

- There is no evidence of increased size of bladder tumours in mice exposed to bifenthrin.
- It is generally considered that size alone is not pathognomonic for increased severity of a tumour.
- Concurrent historical control data show that vascular tumours of the lamina propria of the bladder are common in this strain of mouse.
- The histology of leiomyosarcoma is remarkably different from the vascular lesions in the lamina propria of the bladder observed in this study.
- There is no evidence of a compound-related effect on the incidence of lesions of the liver (hyperplasia, adenoma and carcinoma).

In a published study by Butler, Cohen & Squire (1997), members of the pathology review panel for bifenthrin suggested that the initially reported urinary bladder tumours in bifenthrin-treated male Swiss-Webster mice showed a pattern of both epithelial cells and spindle cells forming irregular vascular channels, which appeared to arise from the region of the bladder and, in some cases, invaded the bladder wall. No metastases were recorded. The tumour (leiomyosarcoma) is rare; however, in this study, it was commonly observed in all groups, but predominantly in males. The histogenesis of the tumour is uncertain, but from its pleomorphic histological features, including smooth muscle and vascularity, it is probably derived from vascular mesenchyme (Butler, Cohen & Squire, 1997).

A summary of pathological evaluations of urinary bladder tumours in bifenthrin-treated male Swiss-Webster mice was prepared by Wells (2006). Based on the conclusions of the experts cited in this review and the European Union criteria for classification of carcinogens, Wells (2006) concluded that bifenthrin does not meet the criteria for classification as a carcinogen.

Rats

In a long-term combined study of toxicity and carcinogenicity, groups of 50 male and 50 female Sprague-Dawley (Tac(SD)fBR) rats were given diets containing bifenthrin (purity 88.35%) at a concentration of 0, 12, 50, 100 or 200 ppm, equal to 0, 0.6, 2.3, 4.7 and 9.7 mg/kg bw per day for males and 0, 0.7, 3.0, 6.1 and 12.7 mg/kg bw per day for females, for 24 months. Prepared diets were sealed in plastic bags and closed containers and stored at room temperature. Stability, homogeneity and dietary concentrations were confirmed analytically. All rats were observed twice a day for mortality and daily for clinical signs and morbidity. Individual body weights and food consumption

were measured weekly for the first 13 weeks of the study and at monthly intervals thereafter. An ophthalmoscopic examination was performed on all animals prior to treatment and at termination of the study. Haematological, clinical chemistry and urinalysis determinations were conducted on 10 animals of each sex per dose at 6, 12, 18 and 24 months (termination) after the treatment. A gross necropsy was conducted on all animals. At termination, organ weights were obtained for brain, kidneys, liver and gonads from 10 animals of each sex per group. Histopathological examination of selected tissues was conducted on all animals in the control and 200 ppm groups. In addition, histopathological examination was performed on the lungs, liver, kidneys, sciatic nerve and spinal cord of animals of the other groups.

Stability, homogeneity and dietary concentrations of bifenthrin were within acceptable ranges. There were no treatment-related differences in survival. At termination, the survival of the males ranged from 34% to 48%, and the survival of the females ranged from 56% to 70%. Tremors were noted in all males at 200 ppm during days 4–28 and in all females at 200 ppm during days 4–30 and days 36–38. At 100 ppm, one female displayed tremors for 3 days (days 3–5). The incidence of tremors decreased during the middle portion of the study and increased towards the termination of the study. Other clinical signs in the 200 ppm males and females included increased incidences of abrasion, alopecia and tail laceration. Mean body weights of females at 200 ppm were significantly lower (↓8–10%, $P < 0.05$ or 0.01) during weeks 13–96. The 200 ppm females also exhibited a significant reduction in total body weight change (↓17%). Mean body weights of the 200 ppm males were decreased (non-significantly) relative to controls from weeks 1 to 92. There were changes in food consumption in male and female rats in various treatment groups, but there was no clear dose–response pattern to the changes in food consumption. No treatment-related changes in the clinical chemistry, urinalysis or ophthalmological examinations were observed. No treatment-related changes in haematological parameters were observed, except for a decrease in red blood cells in the 200 ppm males at 24 months. The decrease in red blood cells was not considered to be toxicologically relevant, as the magnitude of change was small and other red blood cell parameters were not affected. Males at 200 ppm had higher, although not statistically significant, mean liver (↑11%) and kidney (↑28%) weights at 24 months. Males and females at 200 ppm and males at 100 ppm also had higher, although not statistically significant, liver to body weight and kidney to body weight ratios. Three of 28 females at 200 ppm had retinal atrophy, compared with 0 of 40 (controls), 0 of 7 (12 ppm), 0 of 4 (50 ppm) and 0 of 6 (100 ppm). The incidences of pancreatic islet cell adenoma and fibrosarcoma in males at 200 ppm were increased (3/50 versus 1/47 for the controls and 3/50 versus 0/50 for the controls, respectively), but statistical significance was not achieved, and historical control data indicated that these are not rare tumours in this strain. There were no treatment-related findings noted at necropsy. There were no gross or histopathological lesions noted in any animal that could be attributed to the treatment. There were no biologically significant differences between treated groups with respect to histological findings in the sciatic nerve.

The LOAEL is 100 ppm, equal to 4.7 and 6.1 mg/kg bw per day for males and females, respectively, based on the increased incidence of tremors in females. The NOAEL is 50 ppm, equal to 2.3 and 3.0 mg/kg bw per day for males and females, respectively. Under the conditions of this study, there was no conclusive evidence of carcinogenic potential of bifenthrin in rats (McCarty, 1986).

2.4 Genotoxicity

Bifenthrin gave negative responses in various in vitro and in vivo genotoxicity tests except for the test on mouse lymphoma cells at low concentrations and a weak positive response in the unscheduled deoxyribonucleic acid (DNA) synthesis (UDS) assay (Table 10). As the second in vitro test did not replicate the results of the in vitro test and negative findings in the in vivo UDS assay, bifenthrin is

considered to be negative in the UDS assay. Based on the overall weight of evidence, it is concluded that bifenthrin is unlikely to be genotoxic.

Table 10. Results of studies of genotoxicity with bifenthrin

End-point	Test system	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation (Ames test)	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538	75–7500 µg/plate ± metabolic activation in acetone	91.4	Negative	Haworth (1983)
	S. typhimurium strains TA98, TA100, TA1535 and TA1537	8–5000 µg/plate ± metabolic activation in DMSO (experiment 1) 500–4000 µg/plate ± metabolic activation in DMSO (experiment 2)	88.34	Negative	Kennelly (1988)
	S. typhimurium strains TA98, TA100, TA1535 and TA1537 *Escherichia coli* WP2 uvrA	50–5000 µg/plate ± metabolic activation in DMSO	95.7	Negative	Wagner & VanDyke (2006)
Forward mutation	Mouse lymphoma L5178Y cells	0.018–0.24 µg/ml without metabolic activation in acetone 0.0075–0.1 µg/ml with metabolic activation in acetone	88.35	Positive	Kirby (1983)
	Mouse lymphoma L5178Y cells	15.8–500 µg/ml ± metabolic activation in DMSO (test 1) 50–200 µg/ml ± metabolic activation in DMSO (test 2)	88.35	Negative	Kennelly (1986)
CHO/HGPRT mutation assay	CHO/HGPRT (CHO-K_1-BH_4)	250–1000 µg/ml without metabolic activation in DMSO 20–50 µg/ml with metabolic activation in DMSO	88.35	Negative (without metabolic activation) Inconclusive (with metabolic activation)	Thilagar (1984a)
	CHO/HGPRT (CHO-K_1-BH_4)	10–100 µg/ml ± metabolic activation in DMSO	90.58	Negative	Heidemann (1989a)
Chromosomal aberration	CHO cells	100–10 000 µg/ml ± metabolic activation in acetone	88.35	Negative	Thilagar (1984b)
Sister chromatid exchange	CHO cells	1–60 µg/ml ± metabolic activation in DMSO	88.35	Negative	Heidemann (1989b)
Morphological transformation assay	BALB/3T3 mouse embryo cells	3–100 µg/ml without metabolic activation in DMSO	88.35	Negative	Putman (1983a)
UDS	Rat primary hepatocytes	0.01–2 µg/ml in acetone	88.35	Weak positive	Thilagar (1983a)
	Rat primary hepatocytes	0.5–2.5 µg/ml in acetone	88.35	Negative	Thilagar (1983b)
	Rat primary hepatocytes	1–100 µg/ml in acetone	90	Negative	Fautz (1990)

Table 10 (contd)

End-point	Test system	Concentration or dose	Purity (%)	Result	Reference
In vivo					
UDS	Sprague-Dawley rats (male)	10–150 mg/kg bw single gavage dose in acetone (pilot study) 7.5–30 mg/kg bw single gavage dose in corn oil	94.7	Negative	Pant & Sly (2005)
Cytogenetic assay (chromosomal aberrations)	Sprague-Dawley rats (male)	3–30 mg/kg bw per day gavage in corn oil for 5 days	88.35	Negative	Putman (1983b)
Sex-linked recessive lethal assay	*Drosophila melanogaster*	50 and 100 µg/ml in unsalted butter	88.35	Negative	DeGraff & Sky Benson (1984)
Micronucleus formation	ICR mice (male and female, 6–8 weeks old)	50–400 mg/kg bw (single oral dose in corn oil, dose range study) 8.75–35 mg/kg bw (single oral dose in corn oil, definitive study)	94.7	Negative	Krsmanovic & Huston (2005)

CHO, Chinese hamster ovary; DMSO, dimethyl sulfoxide

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study of reproductive toxicity, groups of 25 male and 25 female Sprague-Dawley TAC(SD)fBR rats were given diets containing bifenthrin (purity 88.35%) at a concentration of 0, 30, 60 or 100 ppm (equivalent to 0, 1.5, 3.0 and 5.0 mg/kg bw per day). The amount of test material added to the diet was adjusted to account for purity. Dosing began 8 weeks prior to mating for the F_0 generation and 11 weeks prior to mating for the F_1 generation. F_0 generation animals were bred twice, and F_1 parental animals were selected from the F_{1b} pups. All animals were observed daily for signs of toxicity and twice daily for mortality. Body weights were recorded weekly except during mating for P_1 males. All dams were weighed on gestation days (GDs) 0, 6, 15 and 20 and on lactation days (LDs) 0, 7, 14 and 21. Individual food consumption was measured weekly except during mating (both sexes) and lactation. At birth, the number of pups born, the number of pups born alive or stillborn, sex anomalies and individual body weights were recorded. Body weights were also recorded on LDs 4, 7, 14 and 21. Daily observations were made throughout lactation for mortality and signs of toxicity. On LD 21, F_{1a} and F_{2a} litters were sacrificed and discarded. Shortly thereafter, mating for the F_{1b} and F_{2b} litters was initiated. After all F_{1b} litters were weaned (LD 21), 25 males and 25 females were randomly chosen from each group to continue on study as the F_1 parents. All adults and selected weanlings (10 of each sex per dose) were subjected to a complete necropsy. Organ weights were obtained for brain, heart, liver, kidneys, adrenals and gonads. Microscopic examination of selected tissues was conducted for parental animals in the 0 ppm and the 100 ppm groups and for all weanlings.

There was no treatment-related mortality in either the F_0 or the F_1 generation. Tremors were noted only in females of both generations at 100 ppm, with one F_0 generation rat observed to have clonic convulsions. The tremors occurred during days 9–35 following delivery in the F_0 generation and during days 3–35 following delivery in the F_1 generation. Premating body weights were

comparable to those of the controls for both sexes of both generations at all dose levels. F_0 generation females at 100 ppm had lower mean body weights (↓4%, $P < 0.05$) at week 17 (after gestation and lactation), significantly lower body weights during the first lactation (days 7 and 14) period, and lower body weight gains during the second gestation and lactation periods (statistically significant only on LD 14, ↓5% for body weight and ↓34% for body weight gain, $P < 0.01$). There was no correlation between lower body weight and frequency of tremors. Lower body weights in females at 60 ppm, although not statistically significant, frequently paralleled body weight depression at 100 ppm. There were no clinical signs or effects on body weight at 30 ppm. There were no significant differences in mean body weights between treated F_1 animals and controls. In the high-dose F_0 generation females, there was a statistically significant increase in absolute and relative brain weights. Mean absolute ovary weights were slightly decreased (↓9%, $P < 0.05$, or ↓12%, $P < 0.01$) at 60 and 100 ppm, respectively, in the F_1 parental generation; however, ovary to body weight ratios were unaffected. In the 100 ppm group F_{1b} female progeny, absolute adrenal and heart weights were statistically elevated compared with control values. Significantly elevated absolute ovary weights and ovary to brain weight ratios were also observed in these animals. There were no treatment-related gross or microscopic findings in either adults or progeny. In either the F_0 or F_1 generations, there were no treatment-related effects on reproductive parameters (mating, male fertility, female fertility and gestation indices), and there were no treatment-related gross or microscopic findings in either sex.

The systemic and developmental toxicity LOAEL is 100 ppm, equivalent to 5.0 mg/kg bw per day, based on the incidence of tremors and marginally lower body weights in F_0 and F_1 generation females during gestation and lactation. The systemic and developmental toxicity NOAEL is 60 ppm, equivalent to 3.0 mg/kg bw per day. A reproductive LOAEL was not observed. The reproductive NOAEL is 100 ppm, equivalent to 5.0 mg/kg bw per day (DeProspo, 1986).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 25 pregnant Sprague-Dawley rats were given bifenthrin (purity 88.35%) at a dose of 0, 0.5, 1.0 or 2.0 mg/kg bw per day by gavage in corn oil (dosing volume, 5 ml/1000 g bw) from day 6 to day 15 of gestation, inclusive. An additional group of 25 pregnant rats was administered aqueous aspirin solution at a dosage of 250 mg/kg bw per day by gavage from GD 6 to GD 15 to serve as a positive control. The dosing solutions were prepared daily. All rats were observed twice daily for clinical signs of toxicity, mortality and moribundity. Maternal body weights were recorded on days 0, 6–15 and 20 of gestation. Individual food consumption was calculated on a weekly basis. On day 20 of gestation, all surviving dams were sacrificed and subjected to gross necropsy. Examinations at sacrifice comprised uterine weight, number and positions of implantations, number of corpora lutea in each ovary, individual fetal weights, percentage preimplantation loss, percentage postimplantation loss, and early and late intrauterine deaths. All fetuses were weighed, sexed and examined for external malformations/variations. Approximately half of the fetuses from each litter were preserved in Bouin's fixative, sectioned and examined microscopically using a variation of Wilson's technique for visceral anomalies. The remaining fetuses were necropsied and eviscerated, then stained with Alizarin Red-S and examined for skeletal variations.

There were no deaths during the study, and no significant differences between groups or dose-related trends with respect to mean maternal body weight gains or food consumption were noted. Maternal toxicity was characterized as tremors in 18 of 25 dams at 2.0 mg/kg bw per day during days 10–19. At necropsy, blood clots in the uterus were observed in one rat at 2.0 mg/kg bw per day, and a subcutaneous abdominal mass was observed in one rat in the control group.

There were no group differences or dose-related trends with respect to pregnancy rates, numbers of corpora lutea, implantation sites and resorptions, litter sizes, sex ratios, fetal body weights or viability. Slight fetotoxicity was noted at 2.0 mg/kg bw per day and was characterized as an increased

Table 11. Incidence of hydroureter with and without hydronephrosis in the study of developmental toxicity in rats

Effects	Incidences	Dose group (mg/kg bw per day)				
		0 (vehicle)	0.5	1.0	2.0	0 (positive control)
Hydroureter with hydronephrosis	Fetal incidence	3/146 (2.5)[a]	2/142 (1.41)	3/148 (2.03)	2/141 (1.42)	5/52 (9.61)
	Litter incidence	3/23[b] (13)	2/24 (8.3)	2/24	2/23	
Hydroureter without hydronephrosis	Fetal incidence	0/146 (0)	0/142 (0)	0/148 (0)	5/141 (3.55)	0/52 (0)
	Litter incidence	0/23 (0)	0/24 (0)	0/25 (0)	5/23 (21.74)	
Hydronephrosis without hydroureter	Fetal incidence	0/146 (0)	0/142 (0)	2/148 (1.35)	0/141 (0)	2/52 (3.85)

Data extracted from DeProspo (1984c)

[a] Values are expressed as number of occurrences per number of fetuses examined, and values in parentheses are expressed as percentages.

[b] Values are expressed as number of occurrences per number of litters examined, and values in parentheses are expressed as percentages.

Table 12. Incidence of hydronephrosis and hydroureter in the study of developmental toxicity in rats[a]

Effects	Incidences	Dose (mg/kg bw per day)			
		0	0.5	1.0	2.0
Hydronephrosis	Litter incidence	2/23 (8.7)[a]	2/24 (8.3)	4/25 (16)	2/23 (8.7)
	Fetal incidence	3/146 (2.1)[b]	2/142 (1.4)	5/148 (3.4)	2/141 (1.4)
Hydroureter	Litter incidence	2/23 (8.7)	2/24 (8.3)	3/25 (12)	6/23 (26.1)
	Fetal incidence	3/146 (2.1)	2/142 (1.4)	3/148 (2.0)	7/141 (5.0)

Data extracted from DeProspo (1984c)

[a] Values are expressed as number of occurrences per number of litters examined, and values in parentheses are expressed as percentages.

[b] Values are expressed as number of occurrences per number of fetuses examined, and values in parentheses are expressed as percentages.

fetal and litter incidence of "hydroureter without hydronephrosis" (Table 11). This finding that five fetuses from five different litters had "hydroureter without hydronephrosis" is considered treatment related because it was not present in controls or any of the other exposure groups, including positive controls. Although not statistically significant, the incidence of hydroureter was doubled in the high dose group (Table 12). Also, there were no other treatment-related malformations or variations noted at any dose level. The positive control gave the appropriate responses of increased early resorptions, depressed fetal body weights and external, visceral and skeletal malformations and variations.

The maternal LOAEL is 2.0 mg/kg bw per day, based on the incidence of tremors observed in dams between GD 10 and GD 19. The developmental LOAEL is 2.0 mg/kg bw per day, based on the increased fetal and litter incidence of "hydroureter without hydronephrosis". The maternal and developmental NOAEL is 1.0 mg/kg bw per day (DeProspo, 1984c).

In a second study of developmental toxicity, groups of 25 pregnant Sprague-Dawley rats were administered bifenthrin (purity 95.3%) in the diet at concentrations of 0, 30, 60, 90 or 200 ppm (equal to 0, 2.5, 5.0, 7.4 and 16.3 mg/kg bw per day) from days 6 to 20 of gestation, inclusive. Homogeneity, stability and dietary concentrations were measured analytically. All rats were observed daily for clinical signs of toxicity, mortality and moribundity. Maternal body weights were recorded on days 0, 6, 9, 12, 15, 18 and 20 of gestation. Food consumption was calculated for GDs 0–6, 6–9, 9–12, 12–15, 15–18 and 18–20. On day 20 of gestation, all surviving dams were sacrificed and subjected to gross necropsy. The uterus was removed from each dam and weighed. All fetuses were removed

by caesarean section and examined. The number and distribution of implantation sites, early and late resorptions, live and dead fetuses and corpora lutea were recorded. All fetuses were weighed, sexed and examined for external malformations/variations. Approximately half of the fetuses from each litter were preserved in Bouin's fixative, sectioned and examined microscopically using a variation of Wilson's technique for visceral anomalies. The remaining fetuses were necropsied and eviscerated, then stained for skeletal variations.

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable. No animals died during the study. When compared with concurrent controls, no treatment-related changes were observed in gross pathology, number of corpora lutea, number of implantations, number of live and dead fetuses, number of resorptions, fetal weights, sex ratios or postimplantation losses. At 200 ppm, clinical signs indicative of neurotoxicity were observed. These signs included tremors (22/25), observed from days 9 to 20; hypersensitivity to sound (5/25), observed from days 18 to 20; splayed hind limbs (1/25), observed from days 15 to 20; and piloerection (1/25), observed at day 19. None of these findings were observed in any control animal or any other treatment groups. A negative trend ($P \leq 0.05$) in body weight gain was observed during GDs 6–9, 15–18 and 18–20, with a decrease of 44%, 17% and 14%, respectively, at 200 ppm relative to controls. In addition, a negative trend ($P \leq 0.001$) was observed in adjusted (for gravid uterine weight) body weight gain, with a 22% decrease at 200 ppm when compared with controls. Food consumption was decreased at 200 ppm at the beginning (GDs 6–9) and end (GDs 18–20) of treatment (↓11–12%, relative to controls). In addition, a negative trend ($P \leq 0.05$) was observed during GDs 6–9, 9–12 and 18–20 and for the overall treatment interval (GDs 6–20). Dose-dependent ($P \leq 0.05$ for negative trend) decreases in food consumption were observed; however, the decreases that were noted at doses below 200 ppm did not result in decreased body weight gains and were considered not to be toxicologically important. No treatment-related developmental findings were noted at any dose tested.

The maternal LOAEL is 200 ppm, equal to 16.3 mg/kg bw per day, based on clinical signs and decreased food consumption, body weight gain and adjusted (for gravid uterine weight) body weight gain. The maternal NOAEL is 90 ppm, equal to 7.4 mg/kg bw per day. A developmental toxicity LOAEL was not observed. The developmental toxicity NOAEL is 200 ppm, equal to 16.3 mg/kg bw per day, the highest dose tested (Watt & Freeman, 2001).

Rabbits

In a study of developmental toxicity, groups of 20 pregnant New Zealand White rabbits were given bifenthrin (purity 88.35%) at a dose of 0, 2.67, 4.0 or 8.0 mg/kg bw per day by gavage in corn oil on days 7–19 of gestation, inclusive. An additional group of 20 pregnant rabbits was administered 6-aminonicotinamide in 2% carboxymethyl cellulose via intraperitoneal injection at a dosage level of 3 mg/kg bw on GD 12 to serve as a positive control. Test substance formulations were prepared daily. All rabbits were observed twice per day for mortality or clinical signs of toxicity. Maternal body weights were recorded on days 0, 7 through 19, 21, 28 and 29. Food consumption was not measured. On day 29 of gestation, all surviving does were killed and subjected to gross necropsy. The uterus and ovaries were excised, and the number of corpora lutea on each ovary was recorded. Gravid uteri were weighed and opened, and the location and number of viable and non-viable fetuses, the number of early and late resorptions, and the total number of implantations were recorded. All fetuses were weighed and examined for external malformations/variations. Each fetus was examined viscerally by fresh dissection and the sex determined by the Staples technique. All carcasses were eviscerated and processed for skeletal examination.

There were no treatment-related deaths in the does; however, 10 rabbits died during the study, and 9 of these deaths (including 3 vehicle control animals) were attributed to *Pasteurella multocida*. One death occurred on day 10 of the study from an unknown cause.

Maternal toxicity was characterized at 8.0 mg/kg bw per day as tremors in 17 of 20 rabbits (observed during days 12–23) and twitching of the head and forelimbs in 14 of 20 rabbits (observed during days 13–20). In addition, one rabbit in the 8.0 mg/kg bw per day group displayed clonic convulsions and loss of muscle control during days 17 and 18. At 4.0 mg/kg bw per day, head and forelimb twitching was noted in 4 of 20 rabbits (observed during days 8–16). There were no apparent treatment-related differences in mean body weight gains in the does or pregnancy rates; data excluded those with infection. There were no gross or microscopic findings attributable to exposure to the test material. There were no treatment-related effects on the number of live fetuses, fetal weights, implantations, resorptions, or external, visceral or skeletal malformations and variations. The positive control gave the appropriate responses of increased early resorptions, reduced number of live fetuses, and increased external, visceral and skeletal malformations and variations.

The maternal LOAEL is 4.0 mg/kg bw per day, based on the treatment-related incidence of head and forelimb twitching. The maternal NOAEL is 2.67 mg/kg bw per day. There was no developmental toxicity demonstrated at any dose level. The developmental NOAEL is 8.0 mg/kg bw per day, the highest dose tested (DeProspo, 1984b).

2.6 Special studies

(a) Acute neurotoxicity

Rats

In a study of acute neurotoxicity, four groups of 10 male and 10 female Sprague-Dawley rats were given a single dose of bifenthrin (purity 93.7%) at 0, 10, 35 or 75 mg/kg bw by gavage (undiluted) and observed for the following 14 days. All the rats were evaluated in FOB and motor activity tests on days –7, 1 (6–8 h after dosing; time of peak effects), 7 and 14. All rats were observed before the study start and daily throughout the study for any changes in clinical condition. Body weights were measured on study days 0, 7 and 14. Food consumption was not monitored during the study. Five males and five females per group were perfused in situ for neurohistological examination. Microscopic neuropathological examination was performed on the control and high-dose animals.

Two control males and two control females were removed from the study due to an unspecified dosing error. At 75 mg/kg bw, two females died on day 0, shortly after dosing. No other premature deaths occurred. Body weights and body weight gains were similar in all groups. No treatment-related gross lesions were observed in any animal during necropsy. There were no treatment-related microscopic neuropathological differences in any treated groups relative to controls. The following clinical signs were observed in high-dose males after dosing: decreased faeces, staggered gait, tremors and twitching. High-dose females displayed the following clinical signs following dosing: abdominogenital staining, clonic convulsions, chromorhinorrhoea and tremors. Most of these clinical signs were seen in two females that died. All clinical signs of toxicity were resolved in survivors by study day 2. No findings were observed in the 10 or 35 mg/kg bw animals at any day. The following FOB home cage observations were noted on day 0 at 6–8 h post-dosing: whole-body tremors (1 male and 1 female [later died] versus 0 control); abnormal mobile posture (1 male versus 1 control); uncoordinated movement/ataxia (1 male versus 0 control); splayed hind limbs (1 male versus 0 control); convulsions (2 females [later died] versus 0 control); tense/rigid during handling (1 male, 4 females [2 later died] versus 0 controls); and unusual posture (immobile; 1 female [later died] versus 0 control). Day 0 FOB open field observations included the following: localized spasms/twitching (2 males versus 0 control); whole-body tremors (2 males, 2 females [1 later died] treated versus 0 control); staggered gait (1 male versus 0 control); abnormal posture (mobile; 1 male versus 0 control); uncoordinated movement/ataxia (1 male versus 0 control); splayed hind limbs (1 male versus 0 control); increased activity (1 female versus 0 control); decreased activity (1 female [later died] versus 0

control); convulsions (2 females [1 later died] versus 0 control); walking on toes (1 female versus 0 control); and unusual immobile posture (1 female [later died] versus 0 control). Landing foot splay values were decreased in males during the day 0 FOB (↓15%, $P \leq 0.05$). No treatment-related differences from controls were observed in the FOB assessment in survivors on study days 7 and 14. Mean motor activity was decreased in males on day 0 (↓36%, not statistically significant), whereas motor activity in the females was increased on days 0 and 14 (↑23% and ↑18%, respectively; not statistically significant), although the day 14 increase was not considered biologically significant. No treatment-related findings were observed at 10 or 35 mg/kg bw.

The LOAEL for this study is 75 mg/kg bw, based on mortality (females only), clinical and FOB findings and differences in motor activity. The NOAEL for this study is 35 mg/kg bw (Watt, 1998a).

In a published acute neurotoxicity study involving several compounds, including bifenthrin, by Wolansky, Gennings & Crofton (2006), male Long-Evans rats were exposed to bifenthrin (purity 89.0%) at single gavage doses ranging from 0.03 to 28 mg/kg bw in corn oil (1 ml/kg bw). Control rats received only vehicle. Generally, 6–11 doses per compound and 8–18 rats per dose were treated; for bifenthrin, nine doses were tested. Motor activity was measured for 1 h using 16 figure-eight mazes at the time of peak effects. The time of peak effects was obtained from pilot time course studies using motor activity testing or behavioural observations. The time of peak effects for bifenthrin was determined to be 4 h post-dosing. The motor activity data were analysed using a non-linear exponential threshold activity model (Casey et al., 2004). Bifenthrin induced dose-dependent decreases in motor activity. The threshold dose is defined as an estimate of the highest no-effect level at which treated rats did not display any decreases in motor activity. The ED_{30} is defined as the dose associated with a 30% decrease in motor activity. The threshold dose for bifenthrin was 1.28 mg/kg bw, with 95% confidence intervals of 0.67–1.88 mg/kg bw. The ED_{30} for bifenthrin was 3.21 ± 0.32 mg/kg bw (Wolansky, Gennings & Crofton, 2006).

(b) Delayed neuropathy

Hens

In an acute oral toxicity (LD_{50}) study in female domestic hens (*Gallus gallus domesticus*), bifenthrin (purity 88.3%) was administered as a single dose to 10 animals per dose at 0 (group 1), 1250 (group 2), 2500 (group 3) or 5000 (group 4) mg/kg bw by gavage in corn oil. The treated birds were observed for clinical signs and mortality for 14 days after dosing. For neurotoxicity determination, 10 animals per dose were administered bifenthrin (purity 88.3%) as a single gavage dose of 0 (vehicle control) or 5000 (two groups of bifenthrin treatment) mg/kg bw in corn oil. A separate group of 10 animals received tri-*ortho*-cresyl phosphate (TOCP; positive control) by gavage as a single dose of 500 mg/kg bw in corn oil. The treated birds were observed for clinical signs and mortality for 21 days. On day 22, each group received a single oral gavage dose of the appropriate dose regime as described above. The treated birds were observed for another 21 days for clinical signs and mortality. For the acute lethality study, body weights were measured weekly for 2 weeks prior to treatment and also during a 14-day observation period. For neurotoxicity determination, body weights and food consumption were recorded twice per week. Gross necropsy was performed on all birds at the end of the observation period. Neural tissues (brain, spinal cord, peripheral nerves) were examined microscopically from all birds in the neurotoxicity determination study.

In the acute lethality study, approximately 21 h following dosing, a number of test birds (one in group 2, two in group 3 and three in group 4) showed clinical signs of toxicity, which included unsteadiness or inability to walk, wing-dropping and twitching of the head and neck. Within 48 h following dosing, the observed signs of toxicity were diminishing; by the end of day 3, test birds appeared to have recovered. The few mortalities that occurred following dosing were apparently the

result of bullying by other birds. During the pretreatment period, birds in all groups tended to experience a decrease in body weight. All groups showed decreased body weights during the first week of the treatment, except group 3, which showed slight body weight gain. During the second week of the observation period, all groups gained body weight.

In the neurotoxicity study, approximately 20 h after the first dose, birds dosed with test compound became unsteady, and within a few hours, all test birds were eliciting jerking movements of the head. By the end of day 3, most birds appeared to have recovered. Within approximately 21 h after the second dose, test birds showed varying degrees of unsteadiness, accompanied by jerking head movements or trembling. By the end of day 22, test birds were making violent movements of the head and legs, and 11 out of 18 were unable to stand. Over the next few days, the clinical signs of toxicity were generally less severe, and by day 28, surviving birds appeared to have recovered. No clinical signs of ataxia were noted in the treatment group. One bird from the positive control group (TOCP) demonstrated ataxia following the first dose, and five birds following the second dose. After the first dose, birds treated with test compound showed overall weight losses, followed in general by weight increases over days 3–17. Following the second dose on day 21, marked weight decreases were observed over days 21–24, but, again, test birds generally increased in weight over days 24–43. Food consumption was reduced in the treatment groups. No treatment-related changes in the nerve tissue were found in negative control and bifenthrin-treated groups. The positive control group showed significant degenerative changes in the spinal and peripheral nerves.

In conclusion, the LD_{50} of bifenthrin to adult female hens appeared to be greater than 5000 mg/kg bw. An oral administration of a single dose of bifenthrin at 5000 mg/kg bw followed by a repeated dose after 21 days did not produce any clinical signs of neurotoxicity in adult hens (Roberts et al., 1984).

Rats

In an investigation of possible delayed neurological effects using the tilting plane test, COBS/Wistar rats were dosed with bifenthrin (purity 88.35%) via single oral gavage dose daily for 2 or 5 consecutive days. For the Irwin dose range–finding study, three male rats per dose were dosed orally for 5 consecutive days at 0 (corn oil, group 1), 30 (group 2), 10 (group 3), 3 (group 4) or 1 (group 5) mg/kg bw per day. For the tilting plane test, five male and five female rats per dose were dosed orally for 2 consecutive days at 0 (corn oil) or 30 (group 2) mg/kg bw per day. Detailed clinical observations were performed before dosing, at 2 and 6 h after each dose and at 24, 48, 72, 96 and 120 h after the final oral dose. The tilting plane test was performed every second day from day 2 to day 16. This study was not conducted according to GLP.

In the Irwin dose range–finding study, no notable behavioural or autonomic effects were noted in groups 4 and 5, except for one rat in group 4, which exhibited salivation on day 2. Behavioural and autonomic effects were most notable in groups 2 and 3. No notable effects were recorded at the 2 and 6 h observation period on day 1. All rats in the 30 mg/kg bw per day group exhibited moderate tremor by 24 h post-dosing. All rats in the 30 mg/kg bw per day dose group exhibited tremor, gait, abnormal body carriage and respiratory depression by 2 h after the second dose. In addition, two of the three rats also exhibited signs of central nervous system depression, such as apathy, decreased touch response and paralysis. One rat at this dose level subsequently developed clonic convulsions and died approximately 4 h after dosing. Two animals in the 10 mg/kg bw per day treatment group exhibited signs of possible weak central nervous system stimulation. Prior to dosing on day 3, the fur of surviving rats at 30 mg/kg bw per day had a greasy appearance. During the 2 h post-dose observation period on day 3, these rats were showing effects, which included tremor, paralysis and abnormal body carriage and gait. A similar pattern of effects was seen at 30 mg/kg bw per day on day 4. One rat was found dead at the pre-dose observation on day 5. Another rat died by 24 h after the last dose. No

effects were noted in the 10, 3 or 1 mg/kg bw per day dose groups during the last 7-day observation period after the last dose.

The minimum effective dose at which severe neurological signs such as paralysis were apparent in the Irwin dose range–finding test was 30 mg/kg bw. This dose level was therefore used in the tilting plane test. The results of the tilting plane test indicate that the treatment with bifenthrin at 30 mg/kg bw per day did not produce delayed neurological effects. There was no significant impairment of performance of bifenthrin-treated rats compared with vehicle controls. However, on day 2, some impairment of performance was observed for three rats that received 30 mg/kg bw per day. The toxic effects were most notable after administration of the second dose. Approximately 6 h after the second dose, four of the five female rats showed a stereotypical grooming behaviour. In addition, the fur of these rats had a greasy appearance. No overt toxic signs were recorded in any of the treated males at this stage of the study. Two male and two female rats were found dead 24 h after the second dose. The body weights of the female rats were decreased on days 4–14 of the study. No effects on body weights were observed in male rats. In conclusion, the results of the tilting plane test indicate that treatment with bifenthrin at 30 mg/kg bw per day did not produce delayed neurological effects (Algate et al., 1985).

(c) Short-term studies of neurotoxicity

Rats

In a short-term study of neurotoxicity, groups of male and female Sprague-Dawley rats were given diets containing bifenthrin (purity 93.7%) at a concentration of 0 or 50 ppm (10 rats of each sex per dose) or 100, 200 or 300 ppm (5 rats of each sex per dose) for 28 days. Clinical signs were recorded daily. Body weights were recorded weekly. Low-dose and control animals underwent motor activity testing following 28 days of treatment. All survivors underwent gross necropsies on study day 31.

One 300 ppm female died on day 25. Decreased ($P \leq 0.05$ or 0.01) body weights (8–11%) and body weight gains (16% at day 28) were observed in the 300 ppm males throughout the study. Males receiving doses of 200 ppm and above displayed tremors, twitching, dehydration and unthriftiness. In addition, chromorhinorrhoea, hypersensitivity to sound and splayed hind limbs were observed in the 300 ppm males. No treatment-related clinical signs were observed in males receiving 100 ppm or less. All females receiving doses of 100 ppm or higher displayed tremors, whereas the 200 ppm females displayed twitching and unthriftiness. Abdominogenital staining, chromorhinorrhoea, dehydration, hypersensitivity to sound, splayed hind limbs and unthriftiness were observed in the 300 ppm females. Body weights and body weight gains were also reduced in females at 300 ppm (8.6–10%; gain 25% at day 28), although these decreases were not statistically significant. No treatment-related clinical signs were observed in the 50 ppm females. No gross lesions were noted during necropsy.

The NOAEL for this study was 50 ppm based on tremors at 100 ppm. Based on the results of this study, 0, 50, 100 and 200 ppm dose levels were selected for the 90-day neurotoxicity study in rats (Watt, 1998b).

In a short-term study of neurotoxicity, groups of 10 male and 10 female Sprague-Dawley rats were given diets containing bifenthrin (purity 93.7%) at a concentration of 0, 50, 100 or 200 ppm (equal to 0, 2.9, 6.0 or 11.8 mg/kg bw per day in males and 0, 3.7, 7.2 or 14.6 mg/kg bw per day in females) for 13 weeks. All rats were evaluated in FOB and motor activity tests prior to treatment and following treatment weeks 4, 8 and 13. All rats were observed before the study start and daily throughout the study for any changes in clinical condition. Body weight and food consumption were measured weekly throughout the study. Five male and five female rats from the control group and at the highest dose were perfused in situ and evaluated for microscopic neuropathology. Homogeneity, stability and dietary concentrations were confirmed analytically.

Analysis of the dietary preparations showed that diets were stable at room temperature and the test article was homogeneously distributed in the diet. The analytical data indicated that the variations between nominal and actual dosage to the study animals were acceptable. No treatment-related differences were observed at any dose level in body weights, body weight gains, food consumption, home cage FOB examination, motor activity measurements or gross and neuropathological examinations. No treatment-related findings were observed in the 50 ppm group. At 100 ppm, tremors were observed during clinical examinations in 8 of 10 (28 incidences) males and 10 of 10 (119 incidences) females. Twitching was observed in 4 of 10 (4 incidences) males and 2 of 10 (5 incidences) females. These signs were first observed on day 21 in males and day 20 in females. During the open field portion of the FOB, tremors were observed in all females following 4 weeks of treatment. In addition, females displayed decreased ($P \leq 0.05$) hind limb grip strength during weeks 8 and 13 (22–25%). One female died on day 52 as a result of kidney inflammation; however, this death was not considered treatment related.

At 200 ppm, tremors were observed during clinical examinations in 10 of 10 (311 incidences) males and 10 of 10 (336 incidences) females. Twitching was also observed in 10 of 10 (76 incidences) males and 10 of 10 (96 incidences) females. These signs were first observed on day 8 in males and day 9 in females. During the open field FOB, tremors were observed in all males and females following 4 weeks of treatment. In addition, females displayed increased arousal when compared with concurrent controls (3 treated versus 1 control). Females displayed decreased ($P \leq 0.05$ or 0.01) forelimb grip strength (20–31%) and hind limb grip strength (18–36%) and increased landing foot splay values (21–28%) during weeks 4, 8 and 13. In addition, decreased forelimb grip strength was observed in males at week 4 (27%, not statistically significant), but not at later times.

Under the study conditions utilized, the LOAEL for this study is 100 ppm, equal to 6.0 mg/kg bw per day, based on neuromuscular findings (tremors, changes in grip strength and landing foot splay). The NOAEL is 50 ppm, equal to 2.9 mg/kg bw per day (Freeman, 1998).

(d) Developmental studies of neurotoxicity

Rats

In a range-finding study for the developmental neurotoxicity study, bifenthrin (purity 94.8%) was administered in the diet to 10 female Crl:CD(SD)IGS BR rats per group at concentrations of 0, 50, 65, 80, 100 and 125 ppm (equal to 0, 3.6, 4.6, 6.0, 7.4 and 9.3 mg/kg bw per day, respectively, during gestation; 0, 9.2, 11.7, 14.3, 17.2 and 22.5 mg/kg bw per day, respectively, during lactation) from GD 6 through LD 22. All animals were observed twice daily, and clinical observations were recorded at appropriate intervals. Maternal body weight and food consumption were measured on GDs 0, 6, 9, 12, 15, 18 and 20 and on LDs 1, 4, 7, 11, 14, 17 and 22. Females were allowed to deliver and rear their offspring until postnatal day (PND) 22. On PND 4, litters were culled to yield four males and four females (as closely as possible). Pups were observed for morbidity and moribundity; other clinical observations were also recorded. Body weight was measured on PNDs 1, 4, 7, 11, 14, 17 and 22. Locomotor activity was assessed on one pup of each sex per litter on PND 22. On LDs 5, 11 and 17, milk samples were collected from all dams (following subcutaneous injection of oxytocin) and analysed for the presence of bifenthrin. Blood was collected from five dams per group on LDs 4 and 22, and brain samples were collected on LD 22 from these same animals to analyse for the test material. The culled pups from these five dams were used for blood and brain collection on PND 4; blood and brain samples were pooled by litter. On PND 22, blood and brains were collected and necropsy was performed on one pup of each sex per litter from the five selected litters; the remaining pups and all dams were discarded without examination. Brain weights in dams and offspring were recorded but unreported.

One female in the 50 ppm group delivered on GD 20 and was euthanized along with her pups. All other dams survived to scheduled termination. Slight to moderate whole-body tremors were observed in 8 of 10 dams (beginning as early as LD 5 and extending until LD 22) at 125 ppm. The

finding of whole-body tremors was not observed in any other bifenthrin-treated dose groups. Body weight, food consumption, pregnancy rates and gestation length were unaffected by treatment. No treatment-related clinical effects, macroscopic changes or differences in motor activity were observed in the offspring. Bifenthrin was detected in maternal milk and plasma and in offspring plasma on all sampling days. The highest mean concentration detected in milk occurred on LD 11 for all dietary levels (range of means: 6.01–10.4 ppm). Residue levels increased with increasing dietary concentration in a somewhat linear fashion. Mean maternal plasma levels were almost identical on LDs 4 and 22 and showed a more robust increase with increasing dose than did milk concentrations. Offspring plasma levels were similar to maternal levels, as were the dose–response and time course profiles.

The results of this range-finding study in rats demonstrated that the plasma and milk samples from dams exposed via diet to bifenthrin confirmed that pups were adequately exposed to the test article. Based on whole-body tremors at 125 ppm, the doses of 0, 50, 100 and 125 ppm were selected for a dietary developmental neurotoxicity study of bifenthrin in rats described below (Nemec, 2006a).

In a developmental neurotoxicity study, bifenthrin (purity 94.8%) was administered in the diet to 25 female Crl:CD(SD) rats per dose at dose levels of 0, 50, 100 and 125 ppm (equal to 0, 3.6, 7.2 and 9.0 mg/kg bw per day, respectively, during gestation; 0, 8.3, 16.2 and 20.7 mg/kg bw per day, respectively, during lactation) from GD 6 through LD 21. Maternal animals were checked twice daily for mortality or moribundity and daily for cage-side observations. Individual maternal body weight was recorded on GDs 0, 6, 9, 12, 15 and 20 and on LDs 1, 4, 7, 11, 14, 17 and 21. Food consumption was determined on GDs 0, 6, 9, 12, 15 and 20 and on LDs 1, 4, 7, 11, 14, 17 and 21. An FOB was performed on all dams on GDs 10 and 15 and on LDs 10 and 21. On PND 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory (water maze testing) and neuropathology at termination (PND 72). On PND 21, the whole brain was collected from 10 pups of each sex per group for micropathological examinations and morphometric measurements. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed. Homogeneity, stability and dietary concentrations were confirmed analytically.

The analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable. No dams died during the study. Maternal body weight, body weight gain and food consumption were unaffected by treatment. Tremors were observed during the daily examinations in 8 of 25 females at 100 ppm beginning on LD 14 and in 23 of 25 females in the 125 ppm group beginning on LD 4. In the 100 ppm group, the tremors were graded as slight and resolved in 4 of 8 females after one occurrence; slight tremors were observed in the remaining 4 of 8 females 3–7 times. In the 125 ppm group, the tremors were graded slight to moderate and continued on multiple occasions (2–18 consecutive days) during lactation. Additional test article–related effects consisted of piloerection, noted for up to 4 females in the 125 ppm group during the daily examinations and lactation FOB assessments, and increased mean grooming counts at 125 ppm during the FOB assessments on GD 10 and LDs 10 and 21. Clonic convulsions (limb tremors) and tremors were noted in 2 of 25 and 7 of 25 females, respectively, in the 125 ppm group on LD 10. On LD 21, the number of females with clonic convulsions (limb tremors) and tremors at 125 ppm increased to 10 of 25 and 13 of 25, respectively. Clonic convulsions and tremors were noted in 2 of 23 and 3 of 23 females, respectively, in the 100 ppm group on LD 21. Reproductive performance was unaffected by treatment.

No treatment-related changes were observed at gross necropsy. There were no test article–related effects on the mean number of offspring born per dam, percentage of liveborn and stillborn pups or sex ratio on the day of birth. Offspring postnatal growth and survival were unaffected by maternal

test diet consumption at any dietary concentration. The mean day for reaching sexual maturation (vaginal opening in females and balanopreputial separation in males) was not affected by treatment. No treatment-related changes were observed at gross necropsies of F_1 animals. At 125 ppm, 2 of 20 female offspring had slight tremors during the detailed physical examinations conducted on PND 28, which was 7 days after the last exposure of the dams to the test diet. During the FOB, an increase in the incidence of tremors and clonic convulsions (limb tremors) was observed in males (4/20) at 125 ppm on PND 21. A significant increase in mean grooming counts was noted in females at 100 and 125 ppm on PND 21. No treatment-related changes in forelimb or hind limb grip strength were observed. No treatment-related effects on motor activity, acoustic startle response or learning and memory testing were observed. Brain weight, length and width and macroscopic findings were not affected by treatment. In brain morphometry, a slight increase (3.5%) in the height of the hemisphere (Level 1) that was observed at 125 ppm was not considered toxicologically significant.

The maternal LOAEL for bifenthrin in rats was 100 ppm, equal to 7.2 mg/kg bw per day during gestation and 16.2 mg/kg bw per day during lactation, based on clinical signs of neurotoxicity (tremors, clonic convulsions and increased grooming counts). The maternal NOAEL is 50 ppm, equal to 3.6 mg/kg bw per day during gestation and 8.3 mg/kg bw per day during lactation.

The offspring LOAEL for bifenthrin in rats is 100 ppm, equal to 7.2 mg/kg bw per day during gestation and 16.2 mg/kg bw per day during lactation (maternal dose), based on clinical signs of neurotoxicity (increased grooming counts). The offspring NOAEL is 50 ppm, equal to 3.6 mg/kg bw per day during gestation and 8.3 mg/kg bw per day during lactation (Nemec, 2006b).

(e) Studies on metabolites

No studies on metabolites were submitted by the sponsor, because the metabolites formed in the plants are also formed in animals, and therefore the toxicity of metabolites was assessed in the submitted toxicological studies.

3. Observations in humans

FMC Corporation has been manufacturing bifenthrin technical at FMC's plant in Baltimore, Maryland, USA, since the winter of 1988. Employees are working with bifenthrin about 20% of the time. No significant harmful effects except for mild and temporary paraesthesia (skin tingling) resulting from skin contact were reported in 21 current chemical/raw material operators, supervisors and/or engineers who, since start-up, have worked in the bifenthrin area for any significant period of time. All FMC Baltimore employees, including those who work in the bifenthrin production unit, participate in the medical surveillance programme. Particular attention is given to liver and kidney function, as these would be most likely affected by an overexposure to organic chemicals, including bifenthrin. All employees with potential exposure receive an annual examination, which includes spirometry, audiometric testing, electrocardiogram and a hands-on examination by an occupational health physician, as well as complete haematological, clinical chemistry and urinalysis testing. Based on the medical surveillance programme, FMC concluded that no unexplained or significant changes from the baseline or values falling outside the reference ranges have been noted for employees working in the synthetic pyrethroids business unit, nor have these employees experienced harmful effects as a result of their 14 years of work in this production unit. In addition, an industrial hygiene monitoring programme is also in place at the plant. The results of the industrial hygiene monitoring programme indicated that the airborne levels of the product in the plant are minimal and generally less than the analytical detection limit (O'Grodnick & Niedbalski, 2008).

FMC Corporation emergency calls in 2002 included 58 calls involving formulations containing bifenthrin. Most of these calls were from individuals applying products containing bifenthrin and accidental exposures. The predominant finding was dermal sensations of burning/tingling, which mostly resolved within 24 h. The second most common complaint was eye irritation. No reports

of symptoms from exposure in the general population were found in the open literature search (O'Grodnick & Niedbalski, 2008).

Smith, Thompson & Edwards (2002) developed an extraction and HPLC-ultraviolet method to measure the urinary excretion of 2-methyl-3-phenylbenzoic acid (MPA), a metabolite of bifenthrin, to detect the exposure of workers applying bifenthrin as a termiticide. The results of this biomonitoring of workers applying bifenthrin as a termiticide indicate that the levels of MPA in the urine of workers applying bifenthrin ranged from 1.8 to 31.9 μg/g creatinine (Smith, Thompson & Edwards, 2002). Another article in the literature discusses the development of methods for the analysis of 61 pesticides in human biological matrices. The procedures were used to help diagnose intoxication with bifenthrin (Lacassie et al., 2001).

Comments

Biochemical aspects

In a toxicokinetic study, groups of male and female Sprague-Dawley rats were given bifenthrin labelled with ^{14}C in either the alcohol (phenyl) or acid (cyclopropyl) ring as a single dose at 4 or 35 mg/kg bw or as 14 repeated doses at 4 mg/kg bw per day followed by a single oral dose of radiolabelled bifenthrin at 4 mg/kg bw. There were no significant differences in the results for the different doses and durations. All female rats received alcohol-labelled bifenthrin, and all male rats received acid-labelled bifenthrin. Most of the radiolabel was excreted in the faeces (66–88%) and to some extent in the urine (13–25%) in the first 48 h. Approximately 3% of the administered dose was retained in the body. Fat contained the highest concentrations of bifenthrin-derived radioactivity. In bile duct–cannulated female rats receiving a dose of 2.7 mg/kg bw, mean excretion of radioactivity was 30.0%, 15.0% and 48.7% of the administered dose in the bile, urine and faeces, respectively, 72 h after dosing. Approximately 4.8% of the administered dose was recovered in the gastrointestinal tract, skin and liver in female rats. In male rats at 5.2 mg/kg bw, mean excretion of radiolabel was 18.6%, 10.7% and 24.9% of the administered dose in the bile, urine and faeces, respectively, 72 h after dosing. Approximately 6.3% of the administered dose was recovered in the gastrointestinal tract, skin and liver in male rats. The oral absorption of bifenthrin is estimated to be about 50%. In a study of distribution and bioaccumulation, rats were exposed to bifenthrin for 70 days and 15 days for the depuration phase. Maximum concentrations of radiolabel were detected in the fat (9.62 ppm; half-life 51 days) and skin (1.75 ppm; half-life 51 days). The estimated half-lives were 19 days for liver and 28 days for kidneys. Bifenthrin was metabolized via hydrolysis, oxidation and subsequent glucuronide conjugation. In the faeces, unchanged bifenthrin was the major component (17–45% of the administered radiolabel). Twelve other products derived from hydrolysis and oxidation of the parent compound were also detected in the faeces. Almost no parent compound was detectable in the urine. Nine metabolites derived from hydrolysis and hydrolysis–oxidation products of bifenthrin were detected in the urine.

Toxicological data

Bifenthrin was moderately toxic when administered orally to mice and rats. Data from the studies of acute toxicity in rats suggested that bifenthrin is more toxic when given by gavage in diluted solution (LD_{50} 53 mg/kg bw) than undiluted (melted) (LD_{50} 168 mg/kg bw). In addition, data from the studies of developmental toxicity in rats suggest that bifenthrin is more toxic when given via gavage (the NOAEL for maternal toxicity was 1.0 mg/kg bw) than when given in the diet (the NOAEL for maternal toxicity was 7.4 mg/kg bw). The LD_{50} in rats treated dermally was >2000 mg/kg bw. The LC_{50} in rats treated by inhalation (nose only) was 0.8 mg/l air. Bifenthrin was not irritating to the eyes or skin of rabbits. Bifenthrin was a skin sensitizer as determined by the Magnusson & Kligman (maximization) test in guinea-pigs, but gave a negative response for sensitization in the Buehler test.

Bifenthrin produces characteristic type I pyrethroid neurotoxicity in short- and long-term studies. Clinical signs of neurotoxicity such as tremors were observed in many studies. No reports of histopathological findings in the nervous system were found in the data submitted.

In a 28-day dietary study of toxicity in mice, clinical signs (tremors and convulsions) were observed at 500 ppm, equivalent to 75 mg/kg bw per day, and above, and there were mortalities at 600 ppm and above. The NOAEL was 300 ppm, equivalent to 45 mg/kg bw per day. In a 28-day dietary study of toxicity in rats, tremors were observed at dietary concentrations of 200 ppm, equivalent to 20 mg/kg bw per day, and above. The NOAEL was 100 ppm, equivalent to 10 mg/kg bw per day. In a 90-day dietary study of toxicity in rats, the NOAEL was 50 ppm, equal to 3.8 mg/kg bw per day, on the basis of tremors observed at the LOAEL of 100 ppm, equal to 7.5 mg/kg bw per day.

In a 90-day study of toxicity in dogs fed capsules containing bifenthrin, clinical observations included tremors, ataxia, blinking, mydriasis, nystagmus, lacrimation and polypnoea. The NOAEL was 2.5 mg/kg bw per day on the basis of tremors seen at the LOAEL of 5.0 mg/kg bw per day. In a 1-year study of toxicity in dogs fed capsules, the NOAEL was 1.5 mg/kg bw per day on the basis of an increased incidence of tremors seen at the LOAEL of 3.0 mg/kg bw per day.

The carcinogenic potential of bifenthrin was studied in mice and rats. In mice, the NOAEL was 50 ppm, equal to 7.6 mg/kg bw per day, on the basis of tremors at the LOAEL of 200 ppm, equal to 29 mg/kg bw per day. In this study, males at the highest dose (600 ppm) showed an increased incidence of urinary bladder tumours (leiomyosarcomas). These lesions were re-evaluated by an expert panel of three pathologists, who concluded that the bladder tumours seen in the study in mice were benign, probably vascular in origin, occurred predominantly in males, apparently occurred only in mice and had no relevance for humans. In the study in mice, there was some indication of increased combined incidences of adenoma and adenocarcinoma of the liver (males only) and increased incidences of bronchoalveolar adenomas and adenocarcinomas of the lung in females, but the results of the re-evaluation suggested that these tumour responses were not treatment related.

In a long-term combined study of toxicity and carcinogenicity in rats, tremors were the most prevalent findings in both sexes. At the highest dose of 200 ppm, equal to 9.7 mg/kg bw per day, a slight decrease in body weights was noted, and there was equivocal evidence for decreased food consumption. At the highest dose, retinal atrophy was noted in 28 females but not in males. The NOAEL was 50 ppm, equal to 2.3 mg/kg bw per day, on the basis of tremors seen at the LOAEL of 100 ppm, equal to 4.7 mg/kg bw per day. There were no treatment-related neoplastic findings in rats.

Bifenthrin gave negative responses in various studies of genotoxicity in vitro and in vivo except for a weakly positive response in vitro but not in vivo in the assay for UDS and at low concentrations in a test in mouse lymphoma cells.

The Meeting concluded that bifenthrin is unlikely to be genotoxic.

In view of the lack of evidence for a genotoxic potential in vivo, the absence of carcinogenicity in rats and the lack of relevant carcinogenic effects in mice, the Meeting concluded that bifenthrin 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 (100 ppm, equivalent to 5.0 mg/kg bw per day). The NOAEL for parental systemic toxicity and offspring toxicity was 60 ppm, equivalent to 3.0 mg/kg bw per day, on the basis of marginally reduced body weights in F_0 and F_1 females during gestation and lactation and tremors seen at the LOAEL of 100 ppm, equivalent to 5.0 mg/kg bw per day.

There were two studies of developmental toxicity in rats. In a gavage study in rats, the NOAEL for maternal toxicity was 1.0 mg/kg bw per day on the basis of increased incidence of tremors in 18 out of 25 dams during days 10–19 of gestation, seen at the LOAEL of 2.0 mg/kg bw per day. The NOAEL for developmental toxicity was 1.0 mg/kg bw per day on the basis of increased fetal and litter incidences of hydroureter without hydronephrosis seen at the LOAEL of 2.0 mg/kg bw per day. In

the dietary study of developmental toxicity in rats, the LOAEL for maternal toxicity was 200 ppm, equal to 16.3 mg/kg bw per day, on the basis of clinical signs and decreased food consumption, body weight gains and adjusted (for gravid uterine weight) body weight gains. The NOAEL for maternal toxicity was 90 ppm, equal to 7.4 mg/kg bw per day. The NOAEL for developmental toxicity was 200 ppm, equal to 16.3 mg/kg bw per day, the highest dose tested. In a study of developmental toxicity in rabbits treated by gavage, the NOAEL for maternal toxicity was 2.67 mg/kg bw per day on the basis of treatment-related increases in the incidence of head and forelimb twitching seen at the LOAEL of 4.0 mg/kg bw per day. In this study, no developmental toxicity was observed at doses of up to 8.0 mg/kg bw per day, the highest dose tested.

The Meeting concluded that bifenthrin caused developmental toxicity only at doses that were maternally toxic.

The Meeting concluded that bifenthrin is not likely to be teratogenic to humans.

In a study of acute neurotoxicity in rats given undiluted bifenthrin, the NOAEL was 35 mg/kg bw on the basis of mortality (females only), clinical signs and FOB findings and differences in motor activity observed at the LOAEL of 75 mg/kg bw. In a published study by Wolansky, Gennings & Crofton (2006), male rats were given bifenthrin via gavage as nine doses (8–18 rats per dose) ranging from 0.03 to 28 mg/kg bw in corn oil (1 ml/kg bw), and motor activity was assessed for 1 h during the period of peak effects (4 h after dosing). The data were modelled, and a threshold dose was determined to be 1.28 mg/kg bw. The threshold dose is defined as an estimate of the highest no-effect level at which treated rats did not display any significant decreases in motor activity. In a 90-day study of neurotoxicity in rats, the NOAEL was 50 ppm, equal to 2.9 mg/kg bw per day, on the basis of neuromuscular findings (tremors, changes in grip strength and landing foot splay) observed at the LOAEL of 100 ppm, equal to 6.0 mg/kg bw per day. In a study of developmental neurotoxicity in rats given diets containing bifenthrin, the NOAEL for maternal toxicity was 50 ppm, equal to 3.6 mg/kg bw per day, on the basis of tremors, clonic convulsions and increased grooming counts seen at the LOAEL of 100 ppm, equal to 7.2 mg/kg per day. The NOAEL for offspring toxicity was 50 ppm, equal to 3.6 mg/kg bw per day, on the basis of increased grooming counts seen at the LOAEL of 100 ppm, equal to 7.2 mg/kg bw per day. In studies of delayed neurotoxicity in adult hens and rats, no evidence of delayed neurotoxicity was observed.

On the basis of the available data, the Meeting considered that bifenthrin was neurotoxic.

Workers in a bifenthrin manufacturing plant reported mild and temporary paraesthesia (skin tingling) resulting from skin contact. Of emergency calls received by the manufacturer during 2002 from individuals applying products containing bifenthrin, the most common complaints were dermal sensations of burning/tingling and eye irritation, which mostly resolved within 24 h.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.01 mg/kg bw on the basis of a NOAEL of 1.0 mg/kg bw per day in a study of developmental toxicity in rats (gavage) based on the increased incidence of tremors in dams during days 10–19 of gestation and increased fetal and litter incidences of hydroureter without hydronephrosis seen at the LOAEL of 2.0 mg/kg bw per day, and using a safety factor of 100. This ADI was supported by a threshold dose of 1.3 mg/kg bw in males in a study of acute toxicity in rats treated by gavage and using a safety factor of 100, as well as several other studies, including a 1-year study of toxicity in dogs, a 2-year combined study of toxicity and carcinogenicity in rats and a 90-day study of neurotoxicity in rats, all with NOAELs in the range of 1.5–2.9 mg/kg bw per day.

The Meeting established an acute reference dose (ARfD) of 0.01 mg/kg bw based on a threshold dose of 1.3 mg/kg bw for motor activity in a study of acute toxicity in rats treated by gavage and

using a safety factor of 100. Although this study was conducted with males only, it was considered appropriate, as there was no evidence of sex-specific differences among the data on bifenthrin. This ARfD was supported by the study of developmental toxicity in rats treated by gavage in which the NOAEL of 1.0 mg/kg bw per day was based on the increased fetal and litter incidences of hydroureter without hydronephrosis seen at the LOAEL of 2.0 mg/kg bw per day and which thereby was also protective for developmental effects.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	50 ppm, equal to 7.6 mg/kg bw per day	200 ppm, equal to 29 mg/kg bw per day
		Carcinogenicity	600 ppm, equal to 92 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	50 ppm, equal to 2.3 mg/kg bw per day	100 ppm, equal to 4.7 mg/kg bw per day
		Carcinogenicity	200 ppm, equal to 9.7 mg/kg bw per day[b]	—
	Acute motor activity assessment[c]	Neurotoxicity	Threshold dose, 1.28 ± 0.31 mg/kg bw[d]	3.21 ± 0.32 mg/kg bw[d] (ED_{30})
	Multigeneration study of reproductive toxicity[a]	Parental toxicity	60 ppm, equivalent to 3.0 mg/kg bw per day	100 ppm, equivalent to 5.0 mg/kg bw per day[b]
		Offspring toxicity	60 ppm, equivalent to 3.0 mg/kg bw per day	100 ppm, equivalent to 5.0 mg/kg bw per day[b]
	Developmental toxicity[c]	Maternal toxicity	1.0 mg/kg bw per day	2.0 mg/kg bw per day[b]
		Embryo and fetal toxicity	1.0 mg/kg bw per day	2.0 mg/kg bw per day[b]
	Developmental toxicity[a]	Maternal toxicity	90 ppm, equal to 7.4 mg/kg bw per day	200 ppm, equal to 16.3 mg/kg bw per day[b]
		Embryo and fetal toxicity	200 ppm, equal to 16.3 mg/kg bw per day[b]	—
	Developmental neurotoxicity[a]	Maternal toxicity	50 ppm, equal to 3.6 mg/kg bw per day	100 ppm, equal to 7.2 mg/kg bw per day
		Offspring toxicity	50 ppm, equal to 3.6 mg/kg bw per day	100 ppm, equal to 7.2 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	2.7 mg/kg bw per day	4.0 mg/kg bw per day
		Embryo and fetal toxicity	8.0 mg/kg bw per day[b]	—
Dog	Ninety-day toxicity[c]	Toxicity	2.5 mg/kg bw per day	5.0 mg/kg bw per day
	One-year toxicity[c]	Toxicity	1.5 mg/kg bw per day	3.0 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] The threshold dose is defined as an estimate of the highest no-effect level at which treated rats did not display any decreases in motor activity. The ED_{30} is defined as the dose associated with a 30% decrease in motor activity. From: Wolansky, Gennings & Crofton (2006).

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

0.01 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 bifenthrin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and about 50% oral absorption
Dermal absorption	Moderate, 50%
Distribution	Widely distributed in tissues
Potential for accumulation	Low, no evidence of significant accumulation except fat and skin
Rate and extent of excretion	Approximately 82–90% (70–80% in faeces, 5–25% in urine and 20–30% in bile) within 48 h
Metabolism in animals	Moderate; metabolic pathways include hydrolysis, oxidation and conjugation
Toxicologically significant compounds (animals, plants and environment)	Bifenthrin
Acute toxicity	
Rat, LD_{50}, oral	53.4 mg/kg bw
Rat, LD_{50}, dermal	>2000 mg/kg bw
Rat, LC_{50}, inhalation	0.8 mg/l, dust (4 h exposure, nose only)
Rabbit, dermal irritation	Not an irritant
Rabbit, ocular irritation	Not an irritant
Guinea-pig, dermal sensitization	Sensitizer (Magnusson & Kligman test)
	Not a sensitizer (Buehler)
Short-term studies of toxicity	
Target/critical effect	Tremors
Lowest relevant oral NOAEL	1.5 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	50 mg/kg bw per day (rat)
Genotoxicity	
	Unlikely to be genotoxic
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Tremors
Lowest relevant NOAEL	2.3 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic in rats or mice
Reproductive toxicity	
Reproduction target/critical effect	No toxicologically relevant effects
Lowest relevant reproductive NOAEL	5.0 mg/kg bw per day (rats; highest dose tested)
Developmental target/critical effect	Developmental toxicity only at maternally toxic dose in rats
Lowest relevant developmental NOAEL	2.0 mg/kg bw per day (rats; highest dose tested)

Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	Decrease in motor activity, (threshold dose) 1.28 mg/kg bw (rats)[a]
Short-term study of neurotoxicity	NOAEL: 2.9 mg/kg bw per day (rats)
Developmental neurotoxicity	No neurodevelopmental toxicity observed, NOAEL: 125 ppm, equal to 9.0 mg/kg bw per day (rats), the highest dose tested
Mechanistic data	
	No studies were submitted
Medical data	
	No major effects, and typical symptoms of pyrethroid exposure were reported

[a] The threshold dose is defined as an estimate of the highest no-effect level at which treated rats did not display any decreases in motor activity.

Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Rats, study of developmental toxicity (gavage)	100
ARfD	0.01 mg/kg bw	Rats, acute motor activity assessment	100

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CADUSAFOS

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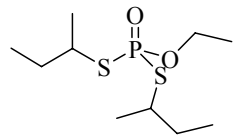
Explanation

Cadusafos (Figure 1) is the International Organization for Standardization (ISO)–approved common name for *S*,*S*-di-*sec*-butyl *O*-ethyl phosphorodithioate (International Union of Pure and Applied Chemistry [IUPAC]) or *O*-ethyl *S*,*S*-bis(1-methylpropyl) phosphorodithioate (Chemical Abstracts Service [CAS]) and has the CAS No. 95465-99-9. Cadusafos is an organothiophosphate insecticide.

Figure 1. Chemical structure of cadusafos



The toxicity of cadusafos was first evaluated by the 1991 Joint FAO/WHO Meeting on Pesticide Residues (JMPR), when an acceptable daily intake (ADI) of 0–0.0003 mg/kg body weight (bw) per day was established on the basis of a no-observed-adverse-effect level (NOAEL) of 0.03 mg/kg bw per day for the inhibition of cholinesterase in plasma and erythrocytes in a multigeneration study in rats and with a safety factor of 100. Cadusafos was reviewed by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues.

In addition to the studies evaluated in 1991, the present Meeting evaluated four new studies: a study of acute neurotoxicity and a short-term study of neurotoxicity in rats, a short-term study of dermal toxicity in rats and an assay for reverse mutations.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

To investigate routes and rates of excretion and tissue distribution of cadusafos, two groups of five male and five female Sprague-Dawley rats were administered single doses of [^{14}C]cadusafos (lot No. NEN 2014-085; radiochemical purity 97%; specific activity 0.74 GBq/mmol; labelled in position 2 of the butyl side-chain) at 20 mg/kg bw by oral gavage in corn oil. All animals were starved for 12 h before dosing. One group was kept in metabolism cages for 3 days to collect expired $^{14}CO_2$, and the other group was used to collect faeces and urine over 7 days; blood samples and organs were collected for analysis of radioactivity. A quality assurance (QA) statement was provided.

During the first 2 h after dosing, all animals showed signs of toxicity, were not moving, were lying on their sides and did not respond to stimulation. All animals recovered and survived until study termination.

The summarized results of the excretion balance study are depicted in Table 1. Renal excretion accounted for 75–79% of the applied dose, and faecal excretion and exhalation each accounted for about 15%. Irrespective of the pathway, excretion was substantially completed within 24 h (Table 2 and Figure 2). In tissues, only 1.7–2.2% of the applied dose was present after 7 days, with highest radioactivity found in liver, adipose tissue and lungs (Table 3). Slightly more radioactivity was found in the brain of males than in the brain of females.

Table 1. Excreted radioactivity within 7 days in rats given a single dose of 20 mg/kg bw of [^{14}C]cadusafos

Excreta	Recovery of radiolabel (% of administered dose ± SD)	
	Males	Females
Urine	74.73 ± 5.33	78.57 ± 6.54
Faeces	15.26 ± 1.99	14.81 ± 8.68
Breath	13.37 ± 0.88	13.66 ± 1.36
Total	111.56	123.62

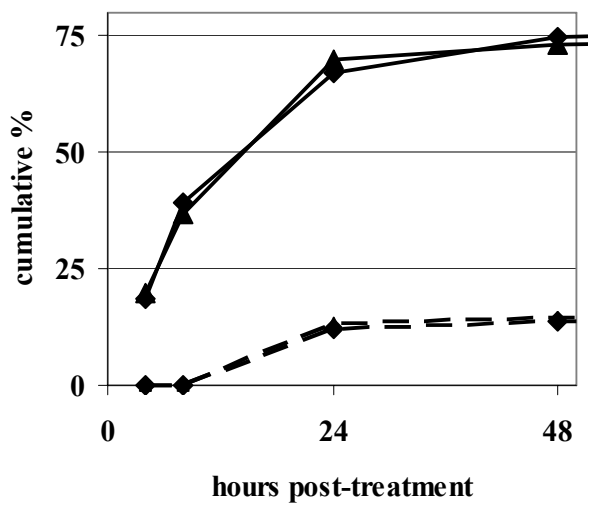
From Selim (1984)
SD, standard deviation

Table 2. Time course of radioactivity excretion in rats given a single dose of 20 mg/kg bw of [^{14}C]cadusafos

Time (h)	Recovery of radiolabel (% of administered dose)			
	Males		Females	
	Urine	Faeces	Urine	Faeces
4	18.39	NA	19.79	NA
8	20.81	0	17.11	0
24	27.73	12.18	32.89	13.39
48	7.83	1.67	3.52	1.34
72	1.39	0.49	0.61	0.23
96	0.95	0.19	0.28	0.11
120	0.57	0.12	0.23	0.1
144	0.31	0.08	0.14	0.04
168[a]	0.61	0.09	0.16	0.05
Total	78.59	14.82	74.73	15.26

From Selim (1984)
NA, not applicable
[a] Including cage rinse.

Figure 2. Cumulative excretion of radioactivity via urine and faeces in rats given a single dose of 20 mg/kg bw of [^{14}C]cadusafos



urine (—); faeces (---); male rats (▲); female rats (♦)

Table 3. Recovery of radiolabel from organs of rats given a single dose of 20 mg/kg bw of [^{14}C]cadusafos

Organ	Recovery of radiolabel			
	Males		Females	
	% of administered dose	ppm	% of administered dose	ppm
Brain	0.007	0.203	0.007	0.121
Heart	0.004	0.184	0.004	0.226
Skeletal muscle	NA	0.131	NA	0.134
Lung	0.014	0.433	0.016	0.475
Adipose	NA	0.564	NA	0.764
Spleen	0.004	0.334	0.003	0.321
Kidney	0.048	0.411	0.017	0.446
Liver	0.189	0.769	0.128	0.621
Blood	NA	0.408	NA	0.439
Plasma	NA	0.051	NA	0.076
Ovaries	NA	NA	0.001	0.392
Uterus	NA	NA	0.001	0.397
Prostate	0.002	0.246	NA	NA
Testes	0.011	0.204	NA	NA
Seminal vesicle	0.003	0.213	NA	NA
Carcass	1.437	0.287	2.022	0.456
Bone	NA	0.158	NA	0.176
Total	1.72	NA	2.2	NA

From Selim (1984)
NA, not applicable; ppm, parts per million

In conclusion, there were no significant differences in excretion or tissue clearance patterns between sexes (Selim, 1984).

To characterize cadusafos pharmacokinetically, three groups of 10 male and 10 female Sprague-Dawley rats were administered single doses of [^{14}C]cadusafos (lot Nos NEN 2014-085 and 2332-016; radiochemical purity >95%; specific activities 0.74 and 0.86 GBq/mmol; labelled in position 2 of the butyl side-chain). One group received the dose of 1 mg/kg bw by oral gavage in corn oil after 12 h of starvation (group 1), and another group received the same dose after 14 days of oral gavage administration of 1 mg/kg bw of non-labelled cadusafos (group 2). The third group was administered a single dose of 0.8 mg/kg bw [^{14}C]cadusafos intravenously (group 3). A group of two male and two female rats served as controls. The study with the first group was repeated with five animals of each sex for analysis of urine and faeces because of low radioactivity recovery in the first experiment. In each of the described groups, half of the animals were used to collect urine and faeces over 7 days and to investigate tissue distribution of radioactivity. The other half of the animals were used to collect the expired radioactivity. The study complied with good laboratory practice (GLP).

Two animals in group 1 had tremors and nine had soft faeces on the first day after treatment. These were the only clinical signs attributable to treatment.

The excretion patterns were very similar between single-dosing and multiple-dosing regimens and intravenous application and also between males and females in terms of the relative amounts in

Table 4. Time course of radiolabel recovery in faeces, urine and carbon dioxide in rats at different dosing regimens

Time (h)	Recovery of radiolabel (% of administered dose)											
	Group 1				Group 2				Group 3			
	Faeces	Urine[a]	CO_2	Total	Faeces	Urine	CO_2	Total	Faeces	Urine	CO_2	Total
Males												
4	—	5.3	5.3	10.6	0	12.7	6.7	19.4	—	30	10.3	40.3
8	—	24.6	3.8	39.0	0	24.7	5.4	49.5	—	31.6	4.1	76.0
12	1.7	23.4	—	64.1	1.2	13.7	—	64.4	0.6	14.6	—	91.2
24	5	14.5	1.5	85.1	5	13.6	2	85.0	4.1	10.9	1.7	107.9
36	0.3	2	—	87.4	1	2.1	—	88.1	0.4	1.8	—	110.1
48	0.1	0.7	0.2	88.4	0.3	1.1	0.3	89.8	0.3	0.6	0.2	111.2
72	0.1	0.4	0.1	89.0	0.2	0.8	0.1	90.9	0.3	0.7	0.1	112.3
96	0	0.3	—	89.3	0.2	0.4	—	91.5	0.1	0.5	—	112.9
120	0	0.2	—	89.5	0.2	0.3	—	92.0	0.1	0.4	—	113.4
144	0	0.2	—	89.7	0.1	0.2	—	92.3	0.1	0.2	—	113.7
168	0	0.1	—	89.8	0.1	2.1[b]	—	94.5	0.1	0.9[b]	—	114.7
% of dose	7.2	71.7	10.9	89.8	8.3	71.7	14.5	94.5	6.1	92.2	16.4	114.7
% of recovery	8.0	79.8	12.1	100.0	8.8	75.9	15.3	100.0	5.3	80.4	14.3	100.0
Females												
4	1.1	11.4	7.3	19.8	—	15.5	8.3	23.8	—	18.9	10.3	29.2
8	—	13.5	4.9	38.2	—	22.4	5.7	51.9	—	32.4	3.8	65.4
12	2.9	16.4	—	57.5	1.3	15.8	—	69	0.5	10	—	75.9
24	7.9	17.5	2.1	85.0	2.2	8.9	2.2	82.3	2	8.9	1.6	88.4
36	0.5	2.1	—	87.6	0.4	2.1	—	84.8	0.2	1.4	—	90.0
48	0.1	0.7	0.5	88.9	0.2	1.3	0.5	86.8	0.4	1.2	0.3	91.9
72	0.1	0.4	0.2	89.6	0.3	0.7	0.2	88	0.3	1	0.1	93.3
96	0.1	0.2	—	89.9	0.1	0.4	—	88.5	0.2	0.6	—	94.1
120	0	0.2	—	90.1	0.3	0.2	—	89	0.1	0.4	—	94.6
144	0	0.1	—	90.2	0.1	0.1	—	89.2	0.1	0.3	—	95.0
168	0	0.1	—	90.3	0.1	3.5[b]	—	92.8	0.1	2[b]	—	97.1
% of dose	12.7	62.6	15	90.3	5	70.9	16.9	92.8	3.9	77.1	16.1	97.1
% of recovery	14.1	69.3	16.6	100.0	5.4	76.4	18.2	100.0	4.0	79.4	16.6	100.0

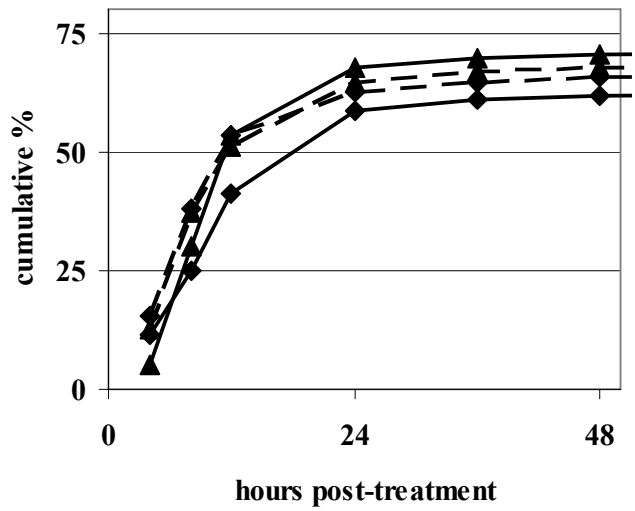
From Puhl (1987)

[a] Including cage rinse.

[b] Including final cage rinse.

urine, faeces and carbon dioxide and also in terms of excretion rates (Table 4 and Figure 3). Of the recovered radioactivity, 70–80% was excreted in the urine, 4–14% in faeces and 12–18% as carbon dioxide. Excretion was practically completed after 24 h. Faecal excretion of males dosed intravenously was 5.3%, being close to the faecal excretion in males dosed orally (8.0% and 8.8%, respectively). In

Figure 3. Cumulative excretion of radioactivity via urine



Single dose group (—); multiple dose group (---); male rats (▲); female rats (♦)

females, faecal excretion was 4.0% in intravenously dosed animals and 14.1% and 5.4%, respectively, in orally dosed animals. Hence, it is concluded that about 5% of the faecal excretion observed in orally dosed animals is attributable to biliary excretion of absorbed material. It is therefore estimated that absorption of cadusafos is close to 100% in males and greater than 90% in females. The tissue distribution of radioactivity was similar for both sexes in all three dosing regimens (Table 5). However, significantly higher levels of radioactivity were found in the lungs of animals of both sexes in the intravenously dosed animals compared with the orally dosed animals. The same effect was seen for male gonads. Additionally, in the kidneys of multiple orally and intravenously dosed males, higher levels of radioactivity were found compared with single orally dosed males. The total radioactivity found in animals 7 days after treatment was 1.0–2.4% of the dose, with highest levels (<0.1 part per million [ppm]) in liver, fat, kidney and lungs (Puhl, 1987).

In a metabolism study, groups of 10 male and 10 female Sprague-Dawley rats were administered either a single oral dose of 1 mg/kg bw (group 1) or 21 mg/kg bw (group 2) or a single intravenous dose of 0.8 mg/kg bw (group 3) of [^{14}C]cadusafos (lot Nos NEN 2014-085 and 2332-016; radiochemical purity >95%; specific activities 0.8 and 0.9 GBq/mmol; labelled in position 2 of the butyl side-chain). A further group was administered oral doses of non-labelled cadusafos at 1 mg/kg bw per day for 14 consecutive days followed by a single oral dose of 1 mg/kg bw of [^{14}C]cadusafos (group 4). All animals were starved for 12 h before [^{14}C]cadusafos was applied. Half of the animals in each group were used to analyse urine and faeces, and the other half were used to analyse expired $^{14}CO_2$. Animals were sacrificed 7 days after treatment. The study complied with GLP guidelines.

Excretion of radioactivity was 63–79% via urine, 5–15% in faeces and 11–17% as carbon dioxide (Table 6). As in a previous study (Puhl, 1987), it is estimated that 90–100% of orally applied cadusafos is absorbed, based on the finding that about 5% of faecal excretion might be attributable to biliary excretion. The tissue distribution of radioactivity was similar for both sexes in all dosing regimens and proportional to the dose applied (Table 7). As in the previous study (Puhl, 1987), significantly higher levels of radioactivity were found in the lungs of intravenously dosed animals of both sexes compared with orally dosed animals. Higher levels of radioactivity

Table 5. Recovery of radiolabel from organs of rats at different dosing regimens

Organ	Recovery of radiolabel											
	Group 1				Group 2				Group 3			
	Male		Female		Male		Female		Male		Female	
	% of dose	ppm	% of dose	ppm	% of dose	ppm	% of dose	ppm	% of dose	ppm	% of dose	ppm
Liver	0.36	0.057	0.19	0.035	0.38	0.067	0.16	0.035	0.37	0.043	0.16	0.023
Fat	0.02	0.033	0.01	0.025	0.02	0.017	0.01	0.021	0.03	0.029	0.01	0.025
Hair	0.01	0.031	0.01	0.033	0.01	0.063	0.01	0.053	0.02	0.041	0.01	0.017
Lung	0.01	0.014	0.01	0.013	0.01	0.024	0.01	0.021	0.04	0.054	0.04	0.055
Kidney	0.02	0.014	0.01	0.016	0.02	0.052	0.02	0.019	0.02	0.046	0.02	0.016
Blood	—	0.013	—	0.016	—	0.016	—	0.016	—	0.024	—	0.025
Skin	0.01	0.012	0.01	0.009	0.01	0.013	0.01	0.012	0.02	0.019	0.01	0.011
Carcass	1.1	0.01	0.96	0.009	1.25	0.011	1.12	0.011	1.8	0.02	1.44	0.011
Pancreas	0	0.008	0	0.011	0	0.012	0	0.013	0.01	0.014	0	0.013
Spleen	0	0.007	0	0.01	0	0.012	0	0.012	0.01	0.012	0	0.012
Brain	0.01	0.007	0.01	0.007	0.01	0.008	0.01	0.008	0.01	0.011	0.01	0.01
Heart	0	0.007	0	0.007	0	0.009	0	0.008	0.01	0.01	0.01	0.01
Bone	0	0.007	0	0.008	0	0.009	0	0.008	0	0.009	0.01	0.009
Gonads	0.01	0.006	0	0.015	0.01	0.009	0	0.016	0.04	0.022	0	0.007
Muscle	0.01	0.006	0.01	0.005	0	0.006	0.01	0.006	0.01	0.009	0.01	0.006
Uterus	NA	NA	0	0.011	NA	NA	0	0.014	NA	NA	0	0.011
Total	1.2		1.03		1.34		1.2		2.39		1.73	

From Puhl (1987)
0, <0.01; —, not calculated; NA, not applicable

Table 6. Total recovery of radiolabel at study termination in rats given single oral or intravenous doses of [^{14}C]cadusafos

Matrix	Recovery of radiolabel (% of administered dose)							
	Group 1		Group 2		Group 3		Group 4	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine	71.56	62.65	74.73	78.57	78.88	76.99	71.74	71.13
Faeces	7.36	12.75	15.26	14.81	5.10	3.81	8.40	4.77
CO_2	10.88	15.02	13.37	13.66	13.98	16.17	14.42	16.79
Tissues	0.46	0.26	0.28	0.18	0.50	0.30	0.47	0.25
Carcass	1.10	0.96	1.44	2.02	1.54	1.44	1.25	1.12
Total	91.36	91.64	105.08	109.24	100.0	98.71	96.28	94.06

From Wu (1988)

Table 7. Recovery of radiolabel from organs of rats given single oral or intravenous doses of [^{14}C]cadusafos

Organ	Recovery of radiolabel (ppm)							
	Group 1		Group 2		Group 3		Group 4	
	Males	Females	Males	Females	Males	Females	Males	Females
Brain	0.007	0.007	0.219	0.189	0.011	0.01	0.008	0.008
Bone	0.007	0.008	0.158	0.176	0.009	0.009	0.009	0.008
Fat	0.033	0.025	0.564	0.764	0.029	0.025	0.017	0.021
Heart	0.007	0.007	0.202	0.226	0.01	0.01	0.009	0.008
Kidney	0.014	0.016	0.411	0.446	0.046	0.016	0.052	0.019
Liver	0.057	0.035	0.769	0.621	0.043	0.023	0.067	0.035
Lungs	0.014	0.013	0.433	0.475	0.054	0.055	0.024	0.021
Muscle	0.006	0.005	0.132	0.134	0.009	0.006	0.006	0.006
Skin	0.012	0.009	—	—	0.019	0.011	0.013	0.012
Spleen	0.007	0.01	0.334	0.321	0.012	0.012	0.012	0.012
Blood	0.013	0.016	0.408	0.439	0.024	0.025	0.016	0.016
Gonads	0.006	0.026	0.665	0.79	0.022	0.018	0.009	0.03
Carcass	0.01	0.009	0.287	0.457	0.012	0.011	0.011	0.011

From Wu (1988)
—, included in carcass

were also seen in male gonads of animals dosed intravenously and orally at the high dose compared with the orally dosed males at the low dose. Additionally, higher levels of radioactivity were found in the kidneys of multiple orally and intravenously dosed males compared with single orally dosed males.

Cadusafos is extensively metabolized. At the low oral dose, only 5.6% of the dose is excreted as parent in the faeces of females (Table 8). At the higher dose, increased levels of parent were also found in the faeces of males. The proposed metabolism of cadusafos starts with cleavage of one of the thiobutyl groups to give butyl mercaptan and *O*-ethyl-*S*-(2-butyl) phosphorothioic acid. *O*-Ethyl-*S*-(2-butyl) phosphorothioic acid can then be cleaved further to give *S*-(2-butyl) phosphorothioic acid or *O*-ethyl phosphorothioic acid (Figure 4). Butyl mercaptan can be further oxidized to methyl *sec*-butyl sulfide, sulfoxide and sulfone and finally to hydroxysulfones. Butyl mercaptan can also be oxidized to butyl sulfonic acid, followed by ethyl and methyl sulfonic acid. Carbon dioxide could be formed from *sec*-butyl mercaptan or the corresponding sulfonic acid. Carbon dioxide could then also be incorporated into urea and other endogenous compounds (Wu, 1988).

1.2 Effects on enzymes and other biochemical parameters

No information was available.

Table 8. Recovery of metabolites in urine and faeces of rats given single oral or intravenous doses of [^{14}C]cadusafos

Metabolite code[a]	Metabolite recovery (% of dose)															
	Group 1				Group 2				Group 3				Group 4			
	Urine		Faeces		Urine		Faeces		Urine		Faeces		Urine		Faeces	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
1	0.5	0.4	1.6	5.6	0.1	1.2	4.2	6.5	0.1	0.4	0	0	0.2	0.1	1.1	0.1
2	10.8	9.7	ND	ND	9.1	9.4	ND	ND	14.6	13.1	ND	ND	8.5	10.4	ND	ND
3	13.6	8.5	0.9	0.1	8.5	9.9	0.4	0.2	17.6	16.4	0.2	0.1	9.5	9.6	1.1	0.2
4	5.3	7.6	ND	ND	7.3	8.6	ND	ND	7.1	8.6	ND	ND	8.1	8.1	ND	ND
5	0.3	0.2	ND	ND	0.4	0.6	ND	ND	0.3	0.4	ND	ND	0.3	0.3	ND	ND
6	2.5	2.6	0	0	4.8	8.5	0.5	0	1.7	1.5	0.2	0	0.9	2.4	0	0.2
7	6.8	3.6	0.8	1.8	4.9	2	2.5	1.8	3.7	3.9	1.1	0.8	3.8	3.9	1.1	0.7
8	2	2.2	ND	ND	3.5	1.5	ND	ND	0.5	0.4	ND	0.4	0.9	2.4	ND	ND
9	12.3	11.5	ND	ND	10.8	11.2	ND	ND	23.9	15.1	ND	1.2	16.4	10.4	ND	ND
10	13.1	12.1	1.4	1.8	16.9	15.9	2	1.7	17.3	10.2	1.1	ND	15.5	14.4	1.3	0.7
11	ND	ND	2	2.7	ND	ND	2.5	3.1	ND	ND	2.1	ND	ND	ND	1.8	1.5
12	0.4	0.2	ND	ND	0.3	0.8	ND	ND	0.3	0.2	ND	ND	0.5	0.2	ND	ND
Total	67.7	58.6	6.7	12.0	66.7	69.8	12.1	13.3	87.3	70.3	4.7	2.5	64.8	62.4	6.4	3.4

From Wu (1988)

F, female; M, male; ND, not detected

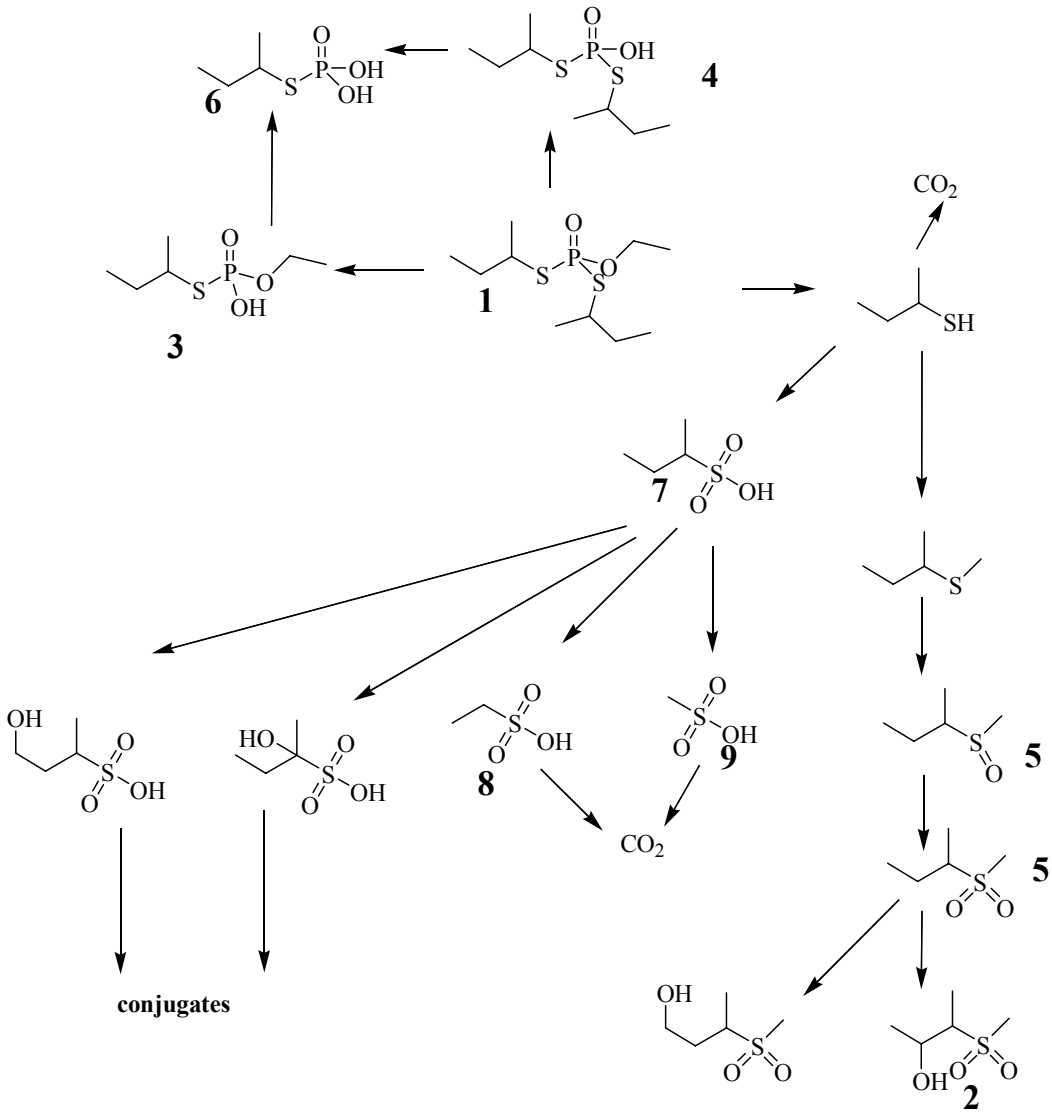
[a] See Table 9 for key to metabolite code.

Table 9. Key to Table 8 and Figure 4

Code in Table 8 and Figure 4	Company code	Name of the compound	CAS No.
1	FMC 67825	Cadusafos; *O*-ethyl-*S*,*S*-di-*sec*-butyl phosphorodithioate	95465-99-9
2	FMC 107619, 107620	Methyl-(3-hydroxy-2-butyl) sulfone (erythro and threo)	102102-60-3 (erythro), 102102-59-0 (threo)
3	FMC 78115	*O*-Ethyl-*S*-(2-butyl) phosphorothioic acid	None found
4	FMC 78135	*S*,*S*-Di-*sec*-butyl phosphorodithioate	878903-76-5
5	FMC 78119, 78121	Methyl-2-butyl sulfoxide, methyl-2-butyl sulfone	70769-71-0 (sulfoxide), 266309-47-1 (sulfone)
6	FMC 78123	*S*-(2-Butyl) phosphorodithioate	44815-22-7
7		*sec*-Butyl-sulfonic acid	16794-12-0
8		Ethyl-sulfonic acid	594-45-6
9		Methyl-sulfonic acid	75-75-2
10		Others	NA
11		Insolubles	NA
12		Methanol	67-56-1

NA, not applicable

Figure 4. Proposed metabolic pathway of cadusafos in rats



Source: Adapted from Wu (1988)

See Table 9 for key to metabolite code.

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with cadusafos are summarized in Table 10.

(a) Oral administration

Groups of 10 male and 10 female Swiss Webster mice were given orally 60, 65, 67, 70, 80 or 90 mg/kg bw (males) and 60, 65, 70, 80, 85 or 90 mg/kg bw (females) of cadusafos (lot No. E 1810:153; purity 94.5%) as a 1% (weight per volume [w/v]) corn oil solution. Animals were observed for 14 days, and a gross necropsy was performed on all animals. A QA statement was provided.

The median lethal doses (LD_{50}s) were 68.4 mg/kg bw in males and 82.1 mg/kg bw in females. The clinical signs were typical effects seen after cholinesterase inhibition (Freeman, 1983b).

Table 10. Acute toxicity of cadusafos

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%)	Reference
Mouse	Swiss Webster	M/F	Oral	68.4 (M) and 82.1 (F)		94.5	Freeman (1983b)
Rat	Sprague-Dawley	M/F	Oral	131.1 (M) and 38.9 (F)		92.5	DeProspo (1986)
Rat	Sprague-Dawley	M/F	Oral	47.5 (M) and 30.1 (F)		94.9	Freeman (1984a)
Rat	Sprague-Dawley	M/F	Oral	80 (M) and 42 (F)		90.31	Freeman (1987b)
Rabbit	New Zealand White	M/F	Dermal	24.4 (M) and 41.8 (F)		94.5	Freeman (1983a)
Rabbit	New Zealand White	M/F	Dermal	12 (M) and 11 (F)		90.31	Freeman (1987a)
Rat	Sprague-Dawley	M/F	Inhala-tion		0.04 (M) and 0.026 (F)	94.9	Dudek (1984)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male

Groups of 10 male and 10 female Sprague-Dawley rats (Tac N(SD)fBR) were given orally 40, 80, 120, 150 or 200 mg/kg bw (males) and 20, 30, 40 or 60 mg/kg bw (females) of cadusafos (lot No. E 3638-179-1; purity 92.5%) as a 1% (w/v) corn oil solution. Animals were observed for 14 days, and a gross necropsy was performed on all animals. The study complied with GLP.

The LD_{50}s were 131.1 mg/kg bw in males and 38.9 mg/kg bw in females. The clinical signs were typical effects seen after cholinesterase inhibition (DeProspo, 1986).

Groups of 10 male and 10 female Sprague-Dawley rats were given orally 30, 40, 50 or 75 mg/kg bw (males) and 20, 25, 30, 40, 45 or 50 mg/kg bw (females) of cadusafos (lot No. E2876-8; purity 94.9%) as a 10% (w/v) corn oil solution. Animals were observed for 14 days, and a gross necropsy was performed on all animals. The study complied with GLP.

The LD_{50}s were 47.5 mg/kg bw in males and 30.1 mg/kg bw in females. The clinical signs were typical effects seen after cholinesterase inhibition (Freeman, 1984a).

Groups of 10 male and 10 female Sprague-Dawley rats (Tac N(SD)fBR) were given orally 50, 70 or 100 mg/kg bw (males) and 30, 35, 40 or 50 mg/kg bw (females) of cadusafos (lot No. PL86-154; purity 90.31%) as a 1% (w/v) corn oil solution. Animals were observed for 14 days, and a gross necropsy was performed on all animals. The study complied with GLP.

The LD_{50}s were 80 mg/kg bw in males and 42 mg/kg bw in females. The clinical signs were typical effects seen after cholinesterase inhibition (Freeman, 1987b).

(b) Dermal application

Groups of five male and five female New Zealand White rabbits were given 20, 22, 24, 25, 27 or 30 mg/kg bw (males) and 15, 20, 25, 30, 33 or 35 mg/kg bw (females) of cadusafos (lot No. E1810:153; purity 94.5%) in a 10% corn oil solution for 24 h by a single application to intact skin followed by a 14-day observation period. The study complied with GLP.

The LD_{50}s were 24.4 mg/kg bw in males and 41.8 mg/kg bw in females. The clinical signs were typical effects seen after cholinesterase inhibition (Freeman, 1983a).

Groups of five male and five female New Zealand White rabbits were given 10, 15 or 20 mg/kg bw (males) and 5, 10 or 15 mg/kg bw (females) of cadusafos (lot No. PL86-154; purity 90.31%) in a 10% corn oil solution for 24 h by a single application to intact skin followed by a 14-day observation period. The study complied with GLP.

The LD_{50}s were 12 mg/kg bw in males and 11 mg/kg bw in females. The clinical signs were typical effects seen after cholinesterase inhibition (Freeman, 1987a).

(c) Exposure by inhalation

Groups of five male and five female Sprague-Dawley rats (Crl:CD(SD)BR) were exposed (whole body) to aerosols of 0.031, 0.033, 0.043, 0.074, 0.136 or 0.404 mg/l of cadusafos (lot No. E2876-8; purity 94.9%; Freeman, 1984a) for 4 h followed by a 14-day observation period. A QA statement was provided.

The median lethal concentrations (LC_{50}s) were 0.04 mg/l in males and 0.026 mg/l in females. The clinical signs were typical effects seen after cholinesterase inhibition (Dudek, 1984).

(d) Dermal and ocular irritation

Cadusafos (lot No. E2876-8; purity 94.9%) was evaluated for its eye-irritating potency in nine New Zealand White rabbits per group at 0.1 and 0.01 ml per animal. The eyes of six animals remained unwashed, and the eyes of three animals were washed 20–30 s after treatment. The study complied with GLP.

Cadusafos is practically not irritating to eyes at 0.01 ml. Animals in the 0.1 ml group died within 2 h after treatment, irrespective of whether the eyes were washed or not (Freeman, 1984d).

Cadusafos (lot No. E2876-8; purity 94.2%) was evaluated for its skin-irritating potency in four New Zealand White rabbits per group at 0.0071 and 0.015 ml per animal. The study complied with GLP.

Cadusafos was not irritating to skin. Four animals at 0.015 ml per animal died within 24 h (Freeman, 1984e).

(e) Dermal sensitization

In a Buehler test, three induction doses, 1 week apart, of 0.01 ml of cadusafos (lot No. E2876-8; purity 94.9%) per animal were applied to the shaved skin of 20 male Hartley guinea-pigs. Two weeks after the last induction, a challenge dose was applied to a different part of the skin of the previously treated animals, as well as to naive animals. Dinitrochlorobenzene was used as a positive control. The study complied with GLP.

There was no evidence of a skin-sensitizing potential of cadusafos, and no clinical signs of intoxication were observed (Freeman, 1984f).

2.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female 30-day-old Swiss Webster mice were fed diets with cadusafos (lot No. E2876-8; purity 92.1%) at concentrations of 0, 0.1, 0.3, 1, 3, 10, 33 and 100 ppm (equal to 0, 0.03, 0.08, 0.21, 0.83, 2.45, 8.0 and 26.25 mg/kg bw per day in males and 0, 0.03, 0.11, 0.33, 1.17, 4.4, 13.3 and 40.0 mg/kg bw per day in females) for 28 days. At the end of the study, blood was collected for the determination of plasma and erythrocyte cholinesterase activities. Gross necropsy was performed, and organ weights were recorded. Brains were collected for the determination of the cholinesterase activity. The study complied with GLP.

Table 11. Cholinesterase activity in mice given diets containing cadusafos for 28 days

Dose (ppm)	Cholinesterase activity								
	Erythrocytes (U/ml)			Plasma (U/ml)			Brain (U/g)		
	Mean	SD	% of control	Mean	SD	% of control	Mean	SD	% of control
Males									
0	1.30	0.61	100	5.89	1.42	100	12.99	0.75	100
0.1	1.16	0.30	89	6.45	1.09	110	13.10	0.61	101
0.3	1.29	0.39	99	6.37	0.72	108	13.01	0.53	100
1	1.22	0.33	94	5.24	1.29	89	13.53	0.64	104
3	1.26	0.13	97	2.71	0.87	46	12.92	0.36	99
10	1.01	0.17	78	0.68**	0.12	12	12.81	0.84	99
33	0.79*	0.12	61	0.23**	0.07	4	8.76**	0.78	67
100	0.72**	0.18	55	0.11**	0.03	2	3.37**	0.50	26
Females									
0	1.77	0.19	100	11.08	0.92	100	12.57	1.21	100
0.1	1.77	0.27	100	10.76	1.40	97	12.84	0.79	102
0.3	1.92	0.35	108	11.30	1.38	102	12.48	1.34	99
1	1.59	0.24	90	9.94	1.22	90	13.02	0.88	104
30	1.50	0.32	85	5.32**	0.83	48	12.92	0.55	103
10	1.20**	0.17	68	1.24**	0.22	11	12.38	0.47	98
33	1.06**	0.24	60	0.39**	0.10	4	10.48**	1.02	83
100	1.09**	0.39	62	0.14**	0.05	1	4.24**	0.64	34

From McCarty (1986)

SD, standard deviation; U, units; * $P < 0.05$; ** $P < 0.01$

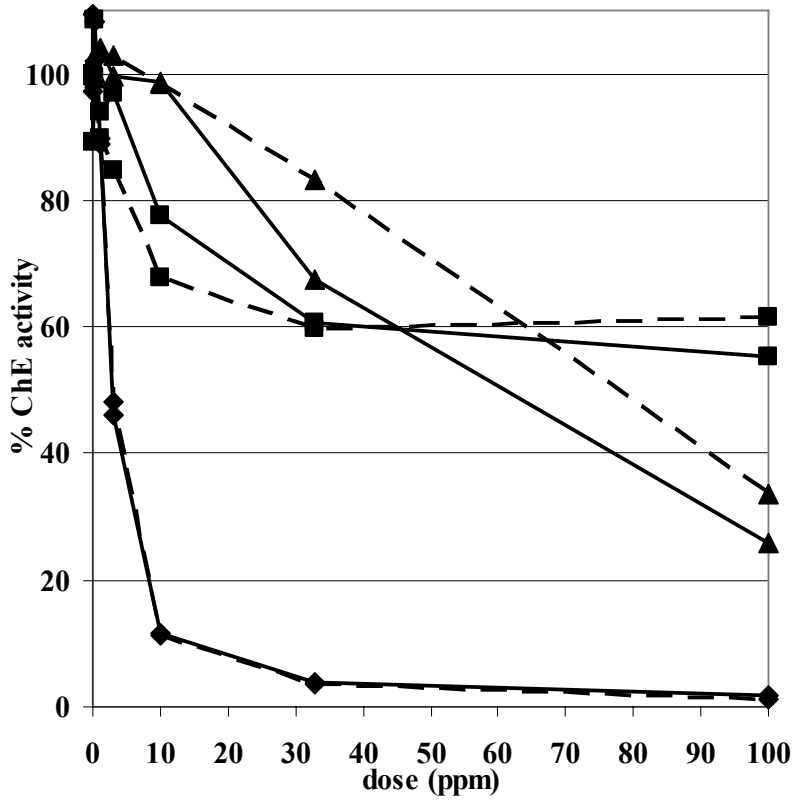
No treatment-related clinical signs or mortalities were observed, and body weight development was comparable between all groups. Additionally, on necropsy, no gross lesions were observed, and organ analysis showed only decreased absolute liver weights and consequently a decreased liver weight to brain weight ratio in the 100 ppm females. Statistically significant inhibition of brain cholinesterase activity was observed at 33 ppm and above in both sexes (Table 11 and Figure 5). Plasma and erythrocyte cholinesterase activities were inhibited statistically significantly at 10 and 33 ppm and above in males and at 3 and 10 ppm and above in females. For both plasma and erythrocytes, a trend in cholinesterase activity inhibition was noted at the next lower doses, although it was not statistically significant.

The NOAEL was 3 ppm, equal to 0.83 and 1.17 mg/kg bw per day in males and females, respectively, based on inhibition of erythrocyte cholinesterase activity at 10 ppm in males, equal to 2.45 and 4.4 mg/kg bw per day in males and females, respectively (McCarty, 1986).

Rats

Groups of 10 male and 10 female 30-day-old rats (strain not specified) were fed diets with cadusafos (lot No. E2876-8; purity 94.2%) at concentrations of 0, 50, 100, 200, 400 or 800 ppm, equal to 0, 4.7, 9.3, 19.6, 39.9 or 56.2 mg/kg bw per day in males and 0, 5.1, 10.7, 21.7, 44.3 or 89.4 mg/kg bw per day in females, respectively, for 28 days. At the end of the study, blood was collected for the determination of plasma and erythrocyte cholinesterase activities. Gross necropsy was performed, and organ weights were recorded. Brains were collected for the determination of the cholinesterase activity. The study complied with GLP.

Figure 5. Relative cholinesterase (ChE) activity in mice given diets containing cadusafos for 28 days



Males (—); females (---); brain (▲), erythrocytes (■); plasma (♦)

At 800 ppm, all males and eight females were dead by days 9 and 11, respectively. From week 1 onwards, body weight gain was decreased in both sexes at 400 ppm and above. Clinical signs as chromorhinorrhoea and chromodacryorrhoea were seen in all groups, and emaciation, tremors, splayed hind legs and abdominogenital staining were seen additionally in the 400 ppm and 800 ppm groups. Lower liver weights and liver weight to brain weight ratios were observed in males at 400 ppm. Most of the other effects on organ weights were either not statistically significant or restricted to females at 800 ppm, with only two surviving animals. Necropsy findings in animals found dead in the 800 ppm group were oral discharge, haemorrhagic intestines and stomach linings and abdominogenital staining. At terminal sacrifice, one male in the 400 ppm group was found moribund with abdominogenital staining and distended intestines. Two other males in this group had abdominogenital staining or swollen prepuce and an extended penis. Statistically significant inhibition of cholinesterase activity was observed at all dose levels in the erythrocytes, plasma and brain of both sexes (Table 12 and Figure 6).

The NOAEL was lower than 50 ppm (the lowest dietary concentration tested), equal to 4.7 and 5.1 mg/kg bw per day in males and females, respectively, based on inhibition of plasma, erythrocyte and brain cholinesterase activities at 50 ppm in both sexes (Rand, 1986).

Groups of 15 male and 15 female 30-day-old Tac(SD)fBR rats were fed diets with cadusafos (lot No. E2876-8; purity 94.2%) at concentrations of 0, 0.1, 0.5, 1, 5 and 800 ppm, equal to 0, 0.008, 0.031, 0.067, 0.330 and 64.42 mg/kg bw per day in males and 0, 0.01, 0.035, 0.076, 0.393 and 73.14 mg/kg bw per day in females, respectively, for 90 days. An additional 10 animals of each sex were

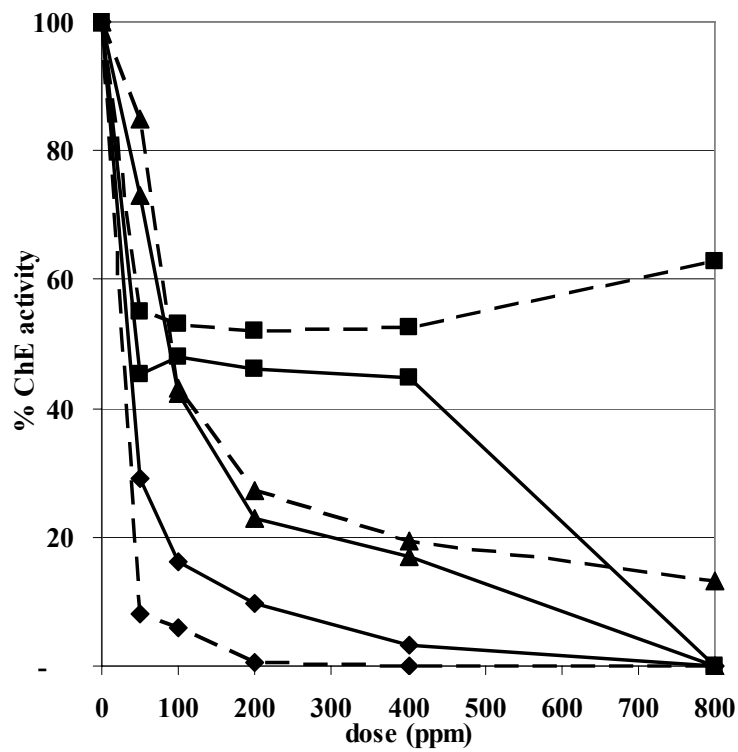
Table 12. Cholinesterase activity in rats given diets containing cadusafos for 28 days

Dose (ppm)	Cholinesterase activity								
	Erythrocytes (U/ml)			Plasma (U/ml)			Brain (U/g)		
	Mean	SD	% of control	Mean	SD	% of control	Mean	SD	% of control
Males									
0	1.63	0.22	100	0.62	0.12	100	12.98	1.76	100
50	0.74**	0.15	45	0.18	0.07	29	9.47**	1.75	73
100	0.78**	0.17	48	0.10**	0.00	16	5.51**	1.35	42
200	0.75**	0.18	46	0.06**	0.05	10	2.98**	0.77	23
400	0.73**	0.13	45	0.02**	0.04	3	2.21**	0.82	17
800	NA	NA	NA	NA	NA	NA	NA	NA	NA
Females									
0	1.75	0.40	100	1.50	0.31	100	11.42	1.21	100
50	0.96**	0.37	55	0.12	0.04	8	9.71**	0.78	85
100	0.93**	0.30	53	0.09*	0.03	6	4.91**	0.84	43
200	0.91**	0.33	52	0.01**	0.03	1	3.12**	0.55	27
400	0.92**	0.29	53	0.00**	0.00	—	2.22**	0.34	19
800	1.10*	0.14	63	0.00**	0.00	—	1.50**	0.00	13

From Rand (1986)

NA, not applicable; SD, standard deviation; U, units; * $P < 0.05$; ** $P < 0.01$

Figure 6. Relative cholinesterase (ChE) activity in rats given diets containing cadusafos for 28 days



Males (—); females (---); brain (▲), erythrocytes (■); plasma (♦)

Table 13. Mean haematological parameters (± standard deviation) in rats given diets containing cadusafos for 90 days

	Dietary concentration (ppm)			% of control
	0	5	800	
Males				
No. of animals	10	10	4	
Erythrocytes (10^{12}/l)	8.9 ± 0.5	9.0 ± 0.6	7.5 ± 0.5**	84.2
Haemoglobin (g/dl)	16.8 ± 0.4	16.6 ± 0.8	13.9 ± 0.4**	83.1
Haematocrit (%)	48.1 ± 1.7	47.6 ± 2.4	39.9 ± 2.2**	82.9
Platelets (10^9/l)	827.8 ± 124.2	889.6 ± 178.0	1321.0 ± 286.2**	159.6
Females				
No. of animals	10	10	2	
Erythrocytes (10^{12}/l)	8.3 ± 0.4	8.3 ± 1.1	7.4 ± 0.3	88.9
Haemoglobin (g/dl)	15.9 ± 0.4	15.9 ± 1.9	13.5 ± 0.6*	84.4
Haematocrit (%)	46.9 ± 2.1	46.2 ± 6.3	41.1 ± 0.5	87.5
Platelets (10^9/l)	980.8 ± 144.9	973.0 ± 141.1	1387.0 ± 157.0**	141.4

From McCarty (1985)
* $P < 0.05$; ** $P < 0.01$

allocated to the control and the 5 ppm groups as a recovery group. They were treated similarly to the respective main groups; at the end of treatment, they were fed control diet for another 28 days.

Animals were observed regularly throughout the study for appearance, behaviour, mortality, body weight development, food consumption, ophthalmic changes, plasma and erythrocyte cholinesterase activities and, at the end of the study, haematological and clinical chemistry parameters. At the end of the study, organ weights were recorded, histology was performed and brain cholinesterase activity was measured. The study complied with GLP.

At 800 ppm, nine males and six females were sacrificed moribund, and two males and five females died in week 2 and another two in weeks 11 and 12, respectively. One female at 5 ppm died on day 97. Animals showed abdominogenital staining, decreased locomotion, splayed hind legs and tremors.

Body weight gain and food consumption were statistically significantly reduced in both sexes at 800 ppm only. Final body weights in this group were reduced by 36% in males and by 25% in females; very few animals were available for statistical analysis. In both sexes, platelets were increased at 800 ppm; in males, haematocrit, haemoglobin and red blood cell counts were significantly reduced (Table 13). At 800 ppm, males had decreased glucose, total protein and globulin levels, and females had decreased total protein, globulin, albumin and calcium and increased urea nitrogen, phosphorus and sodium. Plasma cholinesterase activity was statistically significantly reduced in both sexes at all three time points (30, 60 and 90 days) at 5 and 800 ppm (Table 14). Cholinesterase activity in erythrocytes was reduced in both sexes towards the end of the study at 5 ppm and at all time points in the 800 ppm groups. Brain cholinesterase activity at the end of the study was reduced in females at 5 and 800 ppm and in males at 800 ppm. At the end of the recovery period, erythrocyte, plasma and brain cholinesterase activities in 5 ppm animals did not differ from those of controls.

Males at 800 ppm had statistically significantly increased absolute and relative adrenal weights and decreased absolute and relative to brain heart, kidney, liver and testes weights, but not relative to body weight heart, kidney, liver and testes weights (Table 15). In the recovery group at 5 ppm, males had statistically significantly increased adrenal weights. Females at 800 ppm had lowered absolute and relative ovary weights.

Table 14. Cholinesterase activity in rats given diets containing cadusafos for 90 days

Dose (ppm)	Cholinesterase activity											
	Males						Females					
	30 days		60 days		90 days		30 days		60 days		90 days	
	U/ml or g	%	U/ml or g	%	U/ml or g	%	U/ml or g	%	U/ml or g	%	U/ml or g	%
Erythrocytes (U/ml)												
0	1.3	100	1.9	100	1.6	100	1.2	100	2.2	100	1.4	100
0.1	1.4	108	2.0	104	1.6	99	1.3	105	2.2	99	1.5	109
0.5	1.3	96	1.9	99	1.6	99	1.2	97	2.2	100	1.5	104
1	1.2	94	2.0	104	1.5	93	1.3	105	2.1	94	1.5	104
5	1.0	77	1.7	89	1.3**	78	1.1	88	1.7**	75	1.1**	76
800	0.6**	44	0.8**	40	0.7**	45	0.5**	43	1.3**	56	0.2**	14
Plasma (U/ml)												
0	0.5	100	0.5	100	0.6	100	2.3	100	2.8	100	3.1	100
0.1	0.5	94	0.5	98	0.5	93	2.3	103	2.9	103	3.3	104
0.5	0.5	106	0.5	104	0.6	109	2.4	106	3.0	109	3.6	115
1	0.5	88	0.5	94	0.5	93	2.4	107	2.8	100	3.3	105
5	0.4**	76	0.4*	83	0.5**	81	1.4**	60	1.5**	52	1.7**	54
800	0	0	0	0	0	0	0	0	0	0	0	0
Brain (U/g)												
0	—	—	—	—	10.2	100	—	—	—	—	10.9	100
0.1	—	—	—	—	9.8	97	—	—	—	—	10.9	100
0.5	—	—	—	—	9.6	94	—	—	—	—	10.7	98
1	—	—	—	—	9.5	93	—	—	—	—	10.6	97
5	—	—	—	—	10.0	98	—	—	—	—	10.2*	94
800	—	—	—	—	1.6**	15	—	—	—	—	1.4**	13

From McCarty (1985)
* $P < 0.05$; ** $P < 0.01$

Table 15. Absolute and relative organ weights in rats given diets containing cadusafos for 90 days

Organ	Dietary concentration (ppm)					
	0	0.1	0.5	1	5	800
Males						
Absolute weights (g)						
Body weight	470.9	437.6	451.6	441.9	453.1	304.3
Adrenals	0.054	0.056	0.057	0.057	0.053	0.076**
Brain	2.281	2.253	2.280	2.221	2.274	2.125
Heart	1.564	1.505	1.463	1.516	1.483	1.147**
Kidney	3.443	3.575	3.550	3.435	3.498	2.482**
Liver	17.582	17.193	17.045	16.826	16.658	12.169**

Table 15 (contd)

Organ	Dietary concentration (ppm)					
	0	0.1	0.5	1	5	800
Testes	3.763	3.796	3.776	3.699	3.659	3.151**
Relative to body weight (% of control)						
Adrenals	—	112	110	112	102	218
Brain	—	106	104	104	104	144
Heart	—	104	98	103	99	113
Kidney	—	112	108	106	106	112
Liver	—	105	101	102	98	107
Testes	—	109	105	105	101	130
Relative to brain weight (% of control)						
Adrenals	—	105	106	108	98	151
Heart	—	97	94	100	95	79
Kidney	—	105	103	102	102	77
Liver	—	99	97	98	95	74
Testes	—	102	100	101	98	90
Females						
Absolute weights (g)						
Body weight	272.9	271.6	280.2	281.7	281.6	205.5
Adrenals	0.074	0.072	0.069	0.069	0.071	0.087
Brain	2.042	2.006	2.011	2.006	2.024	1.937
Heart	1.048	1.042	1.057	1.055	1.051	0.917
Kidney	1.933	2.033	2.054	2.086	2.222**	1.975
Liver	9.485	9.758	9.590	9.760	10.365	9.718
Ovaries	0.129	0.130	0.125	0.134	0.133	0.092*
Relative to body weight (% of control)						
Adrenals	—	98	91	90	93	156
Brain	—	99	96	95	96	126
Heart	—	100	98	98	97	116
Kidney	—	106	103	105	111	136
Liver	—	103	98	100	106	136
Ovaries	—	101	94	101	100	95
Relative to brain weight (% of control)						
Adrenals	—	99	95	95	97	124
Heart	—	101	102	102	101	92
Kidney	—	107	108	110	116	108
Liver	—	105	103	105	110	108
Ovaries	—	103	98	106	104	75

From McCarty (1985)
* $P < 0.05$; ** $P < 0.01$

No treatment-related histological findings were seen in animals that survived until study termination. In preterm deaths, signs of atrophy in liver, salivary glands and uterus, acinar cell degranulation of the pancreas, bone marrow hypoplasia, lymphoid depletion of the spleen, lymph nodes and thymus, and germinal epithelial degeneration of testes might be related to the poor general condition of these animals.

The NOAEL in this study was 1 ppm, equal to 0.067 mg/kg bw per day in males and 0.076 mg/kg bw per day in females, based on reduced erythrocyte and brain cholinesterase activities at 5 ppm, equal to 0.330 mg/kg bw per day in males and 0.393 mg/kg bw per day in females (McCarty, 1985).

Dogs

Groups of two male and two female 6-month-old Beagle dogs were administered 0, 0.1 or 1 mg/kg bw per day of cadusafos (lot No. E2876-8; purity 94.9%) 1 h after feeding by capsule for 14 days. Based on the cholinesterase activity inhibition results in erythrocytes and plasma in these groups on study day 4, additional groups at 0.001, 0.005 and 0.02 mg/kg bw per day were set on study 7 days after the 0, 0.1 and 1 mg/kg bw per day groups.

Animals were observed regularly throughout the study for appearance, behaviour, mortality, body weight development, food consumption, ophthalmic changes, plasma and erythrocyte cholinesterase activities and, at the end of the study, haematological and clinical chemistry parameters. At the end of the study, organ weights were recorded, histology was performed and brain cholinesterase activity was measured. The study complied with GLP.

The incidence of soft faeces was increased in animals of both sexes at 0.1 and 1 mg/kg bw per day. There were no evident effects on body weight gain, organ weights, food consumption, clinical chemistry or haematology at any dose level. No overt signs of toxicity were revealed in gross necropsy. Cholinesterase activity was decreased in the plasma of males at 0.1 mg/kg bw per day and above at all time points when compared with both the concurrent controls and the pretest values of the same animals (Table 16). In females, cholinesterase activity in plasma was reduced at 0.02 mg/kg bw per day starting from day 10 and at all time points at higher dose levels when compared with both the concurrent controls and the pretest values of the same animals. No changes in erythrocyte cholinesterase activity were observed for both sexes at any time point or any dose level. No changes in cerebellum and cerebrum cholinesterase activities were observed for both sexes (Table 17).

The NOAEL for erythrocyte and brain cholinesterase activities and other effects was 1 mg/kg bw per day, the highest dose tested (Seely, 1985a).

Groups of four male and four female Beagle dogs 4–6 months of age were administered cadusafos (lot No. E2876-8; purity 94.2%) by capsule at 0, 0.01, 0.03 and 0.09 mg/kg bw per day for 91 days. Animals were observed regularly throughout the study for appearance, behaviour, mortality, body weight development and food consumption. At study day 45 and at the end of the study, haematological and clinical chemistry parameters were measured. At the end of the study, ophthalmological examinations were performed, organ weights were recorded and organs were investigated for gross lesions. Cholinesterase activities in serum and erythrocytes were assayed three times in the pretest phase, at study weeks 1, 2, 4 and 8 and at study termination. Brain cholinesterase activity was determined at study termination. The study complied with GLP.

There were no treatment-related clinical signs, and no effects on food consumption, haematology, clinical chemistry or ophthalmology were observed. No treatment-related effects were evident in gross necropsy. Although statistically not significant, the mean body weights in males, but not in females, were lower in all dosed groups compared with the control animals (Table 18). In the lowest

Table 16. Cholinesterase activity in plasma and erythrocytes at different time points in dogs given cadusafos by capsule for 14 days

Dose (mg/kg bw per day)	Relative cholinesterase activity (%)[a]							
	Males				Females			
	Day 3	Day 7	Day 10	Day 14	Day 3	Day 7	Day 10	Day 14
Plasma, relative to control								
0.001	95	92	92	92	74	73	71	70
0.005	74	81	90	89	78	77	72	73
0.02	93	80	83	83	72	75	**65**	**63**
0.1	**53**	**45**	**44**	**43**	**47**	**34**	**35**	**36**
1	**34**	**25**	**33**	**30**	**24**	**15**	**20**	**25**
Plasma, relative to pretest								
0.001	134	133	127	130	121	116	114	111
0.005	80	90	96	96	125	119	112	112
0.02	110	96	96	97	104	104	**91**	**88**
0.1	**65**	**51**	**50**	**49**	**71**	**49**	**50**	**50**
1	**44**	**29**	**40**	**37**	**45**	**27**	**35**	**43**
Erythrocytes, relative to control								
0.001	116	97	101	68	142	118	94	76
0.005	107	139	123	82	133	119	105	84
0.02	103	123	127	81	156	123	125	99
0.1	97	105	101	122	111	110	100	108
1	97	113	87	104	105	91	113	82
Erythrocytes, relative to pretest								
0.001	163	108	134	116	182	180	131	136
0.005	113	117	123	106	155	166	134	137
0.02	125	118	145	119	171	161	149	153
0.1	93	124	130	125	117	106	104	134
1	89	130	108	102	107	84	112	98

From Seely (1985a)

[a] Bold = treatment related.

Table 17. Cholinesterase activity in cerebrum and cerebellum in dogs given cadusafos by capsule for 14 days

Dose (mg/kg bw per day)	Cholinesterase activity (% of control)			
	Males		Females	
	Cerebrum	Cerebellum	Cerebrum	Cerebellum
0.001	144	78	103	114
0.005	269	108	136	154
0.02	201	116	136	107
0.1	187	110	110	124
1	204	108	98	148

From Seely (1985a)

Table 18. Body weights in dogs given cadusafos by capsule for 91 days

Week	Body weight (% of control)					
	Males			Females		
	Dose (mg/kg bw per day)					
	0.01	0.03	0.09	0.01	0.03	0.09
−1	100	98	98	97	101	100
0	99	99	97	100	103	101
1	100	96	97	100	102	101
2	102	99	97	103	104	102
3	99	97	95	101	103	99
4	100	96	96	102	106	101
5	100	96	96	101	104	99
6	98	94	95	101	104	98
7	96	94	93	100	106	98
8	94	92	90	100	106	99
9	96	94	94	100	107	98
10	94	93	91	98	105	98
11	94	94	93	99	106	98
12	94	93	92	100	105	98
13	93	93	93	99	105	99

From Seely (1985b)

Table 19. Mean absolute and relative testes weights (± standard deviation) in dogs given cadusafos by capsule for 13 weeks

Dose (mg/kg bw per day)	Body weight (kg)	Brain weight (g)	Testes weight (g)	Testes weight/body weight (×1000)	Testes weight/brain weight
0	10.850 ± 0.661	77.294 ± 6.462	19.226 ± 0.712	1.772 ± 0.146	0.249 ± 0.013
0.01	10.200 ± 0.638	77.620 ± 4.290	17.060 ± 1.477	1.673 ± 0.113	0.220 ± 0.009
0.03	10.125 ± 0.826	75.522 ± 6.952	13.906 ± 1.879*	1.373 ± 0.255*	0.184 ± 0.033*
0.09	10.050 ± 0.300	76.756 ± 3.896	14.415 ± 1.481*	1.434 ± 0.176	0.188 ± 0.024*

From Seely (1985b)
* $P \leq 0.05$

dose group, at 0.01 mg/kg bw per day, the effect is observable only from study week 5 onwards. Food consumption was not affected in any dose group. In males at 0.03 and 0.09 mg/kg bw per day, absolute and relative testes weights were statistically significantly decreased (Table 19). The company states that this effect might not be treatment related because the weights of testes in historical control animals (age 25–36 weeks) are 10.97 ± 5.11 g, and sperm at microscopic examination were normal. Moreover, the effect did not occur in a second 13-week study (Dalgard, 1988), designed to compare two different batches of cadusafos (see below).

Plasma cholinesterase activity was markedly decreased in both sexes at 0.03 and 0.09 mg/kg bw per day at all time points (Table 20). No effect on either the erythrocyte cholinesterase activity (Table 20) or the brain cholinesterase activity (Table 21) was observed in both sexes.

Table 20. Cholinesterase activity in plasma and erythrocytes at different time points in dogs given cadusafos by capsule for 91 days

Dose (mg/kg bw per day)	Relative cholinesterase activity (%)									
	Males					Females				
	Week 1	Week 2	Week 4	Week 8	Term	Week 1	Week 2	Week 4	Week 8	Term
Plasma, relative to control										
0.01	89	85	82	81	78	94	90	88	88	85
0.03	73	75	77	63	71	65	69	71	70	76
0.09	46	47	45	50	44	55	53	51	49	50
Plasma, relative to pretest										
0	103	106	104	104	98	99	103	101	101	97
0.01	94	92	88	87	79*	91	91	88	88	81
0.03	67*	71*	71*	59*	62*	60*	66*	67*	65*	68*
0.09	44*	47*	44*	49*	40*	49*	49*	47*	44*	43*
Erythrocytes, relative to control										
0.01	97	92	97	80	95	102	104	106	111	106
0.03	103	97	94	63	92	98	94	103	107	99
0.09	100	97	93	82	93	102	75	103	104	104
Erythrocytes, relative to pretest										
0	100	106	105	113	101	107	111	97	97	106
0.01	102	102	106	94	100	111	116	104	109	113
0.03	106	105	101	73*	95	105	104	99	103	105
0.09	103	106	100	96	96	108	83*	100	100	109

From Seely (1985b)
Term, terminal; * $P \leq 0.05$

Table 21. Cholinesterase activity in cerebrum and cerebellum in the 91-day dog study

Dose (mg/kg bw per day)	Cholinesterase activity (% of control)			
	Males		Females	
	Cerebrum	Cerebellum	Cerebrum	Cerebellum
0.01	90	105	103	107
0.03	100	79	108	115
0.09	102	98	116	136

From Seely (1985b)

The NOAEL was 0.01 mg/kg bw per day, on the basis of decreased mean testes weights at 0.03 mg/kg bw per day and above (Seely, 1985b).

In a study to compare an old lot of cadusafos with a new lot, groups of four male and four female Beagle dogs 4–5 months of age were administered 0, 0.001, 0.01 and 0.1 mg/kg bw per day of cadusafos of either lot No. E2876-8 (purity not given) or lot No. 2445-148 (purity not given)

by capsule for 91 days. Animals were observed regularly throughout the study for appearance, behaviour, mortality, body weight development and food consumption. At the end of the study, organ weights were recorded, and organs were investigated for gross lesions. Cholinesterase activities in serum and erythrocytes were assayed in the pretest phase, at study weeks 2, 3, 5 and 9 and at study termination. Brain cholinesterase activity was determined at study termination. The study complied with GLP.

No clinical signs of toxicity were observed. Plasma cholinesterase activity, but not erythrocyte or brain cholinesterase activity, was decreased at 0.1 mg/kg bw per day. There was no effect on body weight or organ weight development in any dose group. The effect on testes weights observed in a previous study (Seely, 1985b) was no longer observed up to the highest dose tested.

The NOAEL, based on plasma cholinesterase activity, was 0.01 mg/kg bw per day, and the NOAEL for all other parameters, including erythrocyte and brain cholinesterase activity, was 0.1 mg/kg bw per day, the highest dose tested (Dalgard, 1988).

Groups of four male and four female 4-month-old Beagle dogs were administered 0, 0.0002, 0.001, 0.005 or 0.02 mg/kg bw per day of cadusafos (lot No. E2876-8; purity 93.6%) daily by capsule for 52 weeks. The dose was usually administered approximately 1 h following feeding. Animals were observed regularly throughout the study for appearance, behaviour, mortality, body weight development and food consumption. At study initiation, at 6 months and prior to study termination, clinical chemistry, haematological and urinalysis parameters were measured. Cholinesterase activities in serum and erythrocytes were assayed in the pretest phase, at study months 1, 3 and 6 and at study termination. Brain (cerebrum and cerebellum) cholinesterase activities were determined at study termination. Organ weights were determined, and a full histopathological examination was performed. All animals were examined ophthalmologically before study initiation and at study termination. The study complied with GLP.

There were no treatment-related clinical signs, and body weight development and organ weights were not affected. Clinical chemistry parameters were comparable among all groups. Histologically, no effect on organs was observed. Erythrocyte counts, haemoglobin and haematocrit levels were increased in all dosed females at the terminal sacrifice, but not at 6 months; a dose–response relationship is not evident (Table 22). In males, no effect on haematology was observed. Erythrocyte and brain cholinesterase activities were not inhibited at any dose level or time point in both sexes

Table 22. Means and standard deviations of haematological parameters in female dogs given cadusafos by capsule for 52 weeks

Dose (mg/kg bw per day)	Pretest	% of control	6 months	% of control	Terminal	% of control
Red blood cell count (10^6/mm^3)						
0	6.28 ± 0.406	—	7.255 ± 0.562	—	6.77 ± 0.618	—
0.0002	6.455 ± 0.265	103	7.523 ± 0.411	104	7.7 ± 0.278*	114
0.001	6.915 ± 0.399	110	7.158 ± 0.419	99	7.653 ± 0.12*	113
0.005	6.185 ± 0.501	98	7.363 ± 0.331	101	7.695 ± 0.207*	114
0.02	6.535 ± 0.526	104	7.478 ± 0.213	103	7.445 ± 0.286*	110
Haemoglobin (g/dl)						
0	13.95 ± 1.201	—	16.125 ± 0.903	—	15.525 ± 1.187	—
0.0002	14.4 ± 0.523	103	16.65 ± 0.926	103	17.7 ± 0.455*	114
0.001	15.15 ± 0.545	109	15.6 ± 0.356	97	17.375 ± 0.793*	112
0.005	13.975 ± 0.954	100	16.2 ± 0.594	100	17.7 ± 0.529*	114

Table 22 (contd)

Dose (mg/kg bw per day)	Pretest	% of control	6 months	% of control	Terminal	% of control
0.02	14.325 ± 1.184	103	16.575 ± 0.974	103	17.175 ± 0.435*	111
Haematocrit (%)						
0	42.25 ± 3.573	—	47.35 ± 2.901	—	44.85 ± 3.148	—
0.0002	41.775 ± 1.546	99	49.475 ± 2.898	104	52.35 ± 0.686*	117
0.001	44.15 ± 1.034	104	47.225 ± 1.546	100	51.1 ± 1.903*	114
0.005	41.00 ± 2.632	97	48.35 ± 2.327	102	51.65 ± 1.42*	115
0.02	43.45 ± 3.771	103	48.525 ± 2.504	102	50.8 ± 0.757*	113

From Shellenberger (1986)
* $P \leq 0.05$

(Tables 23 and 24). Plasma cholinesterase activity was inhibited statistically significantly in females at study termination in the 0.005 mg/kg bw per day group and at all time points at the highest dose level. Although not statistically significant, a tendency to inhibition of plasma cholinesterase activity was also observed for the other time points in females at 0.005 mg/kg bw per day and for males at 0.02 mg/kg bw per day.

The inhibition of plasma cholinesterase activity in females at 0.005 and 0.02 mg/kg bw per day was not considered to be toxicologically relevant. Therefore, the NOAEL was 0.02 mg/kg bw per day, the highest dose tested (Shellenberger, 1986).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 60 male and 60 female 30-day-old Swiss Webster (Tac(SW)fBR) mice were fed diets containing 0, 0.1, 0.5, 1 or 5 ppm cadusafos (lot No. E2876-8; purity 93.42%) for 97 weeks (males) or 94 weeks (females). The concentrations of cadusafos in food were equal to doses of 0, 0.014, 0.072, 0.141 and 0.705 mg/kg bw per day in males and 0, 0.020, 0.097, 0.189 and 1.008 mg/kg bw per day in females.

Animals were observed regularly for appearance, behaviour and clinical signs, and body weight and food consumption were recorded weekly within the first 13 weeks and at 4-week intervals thereafter. Blood for haematology and for plasma and erythrocyte cholinesterase activity inhibition assay was collected prior to the study, at 1, 6, 12 and 18 months and at study termination. At 12 months, 10 animals of each sex per group were killed for an interim examination. Both the interim kill animals and the animals sacrificed at study termination were subjected to a full necropsy, organ weights were recorded and histopathology was performed. The study complied with GLP.

The survival curves, food consumption, body weight development and organ weights were comparable among all groups. In female mice of the 5 ppm group, lymphocyte counts were statistically significantly increased at 18 months (Table 25). This is probably toxicologically not relevant, because the lymphocyte counts in the control animals were unusually low at this time point. In male animals, for unknown reasons, lymphocyte counts were unusually low in the control and the 5 ppm animals. In both sexes at 5 ppm, statistically significant decreases in plasma and erythrocyte cholinesterase activities at all time points were observed (Table 26). In females of the 5 ppm group at study termination, the effect was no longer statistically significant. In

Table 23. Cholinesterase activities in plasma and erythrocytes at different time points in dogs given cadusafos by capsule for 52 weeks

Dose (mg/kg bw per day)	Relative cholinesterase activity (%)									
	Males					Females				
	Time point (months)									
	Pretest	1	3	6	12	Pretest	1	3	6	12
Plasma, relative to control										
0.0002	111	106	111	105	112	98	91	95	91	87
0.001	112	108	111	115	109	107	86	102	89	87
0.005	120	110	114	108	104	105	82	82	74	71
0.02	131	93	99	91	80	90	69	71	64	59
Plasma, relative to pretest										
0	100	101	99	99	100	100	102	98	110	113
0.0002	100	97	99	94	101	100	94	95	102	99
0.001	100	98	99	102	98	100	81	93	91	92
0.005	100	93	95	89	87	100	80	77	78	77*
0.02	100	72	75	68	61	100	78*	77*	78*	74*
Erythrocytes, relative to control										
0.0002	100	102	102	106	99	104	102	103	100	104
0.001	99	105	101	101	101	102	102	99	96	103
0.005	102	101	105	104	101	104	93	103	98	100
0.02	103	98	107	99	101	103	108	100	94	100
Erythrocytes, relative to pretest										
0	100	104	108	99	98	100	99	113	102	97
0.0002	100	107	110	106	97	100	96	112	97	96
0.001	100	110	110	102	100	100	99	110	96	98
0.005	100	103	111	102	97	100	88	111	95	93
0.02	100	99	112	95	96	100	103	109	93	94

From Shellenberger (1986)
* $P \leq 0.05$

Table 24. Cholinesterase activity in cerebrum and cerebellum in dogs given cadusafos by capsule for 52 weeks

Dose (mg/kg bw per day)	Cholinesterase activity (% of control)			
	Males		Females	
	Cerebrum	Cerebellum	Cerebrum	Cerebellum
0.0002	136	95	107	99
0.001	113	101	110	97
0.005	99	106	110	85
0.02	124	110	118	94

From Shellenberger (1986)

Table 25. Lymphocyte and segmented leukocyte counts in mice given diets containing cadusafos for 94/97 weeks

Dietary concentration (ppm)	Lymphocyte and leukocyte counts (counts per 100 white blood cells ± standard deviation)					
	12 months		18 months		Termination	
	Lymphocytes	Segmented leukocytes	Lymphocytes	Segmented leukocytes	Lymphocytes	Segmented leukocytes
Males						
0	33.3 ± 18.24	65.8 ± 17.76	40.4 ± 26.31	57.5 ± 25.2	70.2 ± 29.06	29.1 ± 29
5	45.0 ± 21.9	54.2 ± 21.7	47.7 ± 26.2	49.8 ± 26.0	79.2 ± 11.8	20.5 ± 11.8
% of control	135	82	118	87	113	70
Females						
0	73.1 ± 9.72	25.8 ± 9.51	59.5 ± 14.04	39.4 ± 14.03	68.4 ± 15.78	30.9 ± 15.76
5	71.9 ± 13.4	27.2 ± 12.4	72.5 ± 10.6*	26.2 ± 10.2*	72.8 ± 21.3	26.8 ± 20.8
% of control	98	105	122	66	106	87

From McCarty (1987)
* $P \leq 0.05$

Table 26. Cholinesterase activity in plasma and erythrocytes in mice given diets containing cadusafos for 94/97 weeks

Dietary concentration (ppm)	Cholinesterase activity (% of control)										
	Males					Females					
	Time point (months)										
	1	6	12	18	Terminal	1	6	12	18	21	Terminal
Plasma											
0	100	100	100	100	100	100	100	100	100	100	100
0.1	99	115	105	97	93	111	90	85	99	96	96
0.5	96	113	108	94	92	110	101	97	91	98	76
1	98	102	106	107	103	97	87	80	90	93	81
5	24**	37**	36**	28**	30**	32**	31**	35**	41**	42**	40**
Erythrocytes											
0	100	100	100	100	100	100	100	100	100	100	100
0.1	100	103	103	101	103	116	97	90	96	84*	105
0.5	104	104	102	100	97	101	96	107	84	97	118
1	106	106	111	104	106	105	98	100	100	90	105
5	74**	86	77**	75**	76**	80**	77**	77**	72**	68**	90

From McCarty (1987)
* $P \leq 0.05$; ** $P \leq 0.01$

the brains of males and females of the 5 ppm group, a statistically not significant decrease in cholinesterase activity was identified (Table 27). This effect is considered treatment related in males, as individual brain cholinesterase activity data of males at the terminal sacrifice suggest a trend to more animals with lower cholinesterase activity, although the mean value for this group is statistically not significant (Table 28). Adrenal cortical atrophy was increased in

Table 27. Brain cholinesterase activity at the interim kill and at study termination in mice given diets containing cadusafos for 94/97 weeks

Dietary concentration (ppm)	Cholinesterase activity (% of control)			
	Males		Females	
	Interim	Terminal	Interim	Terminal
0.1	100	102	101	97
0.5	100	99	100	96
1	103	100	98	95
5	101	87	98	92

From McCarty (1987)

Table 28. Individual brain cholinesterase activity at study termination in mice given diets containing cadusafos for 94/97 weeks[a]

	Cholinesterase activity (U/g)									
	Males					Females				
	Dietary concentration (ppm)									
	0	0.1	0.5	1	5	0	0.1	0.5	1	5
	11.9	12.2	11.7	11.0	6.4	12.2	11.2	11.9	11.3	11.6
	12.4	12.3	12.1	12.1	7.9	12.6	12.2	11.9	11.5	11.7
	12.6	12.6	12.2	12.4	9.3	12.7	12.4	12.3	11.8	11.8
	12.8	12.9	12.2	12.6	10.9	12.8	12.9	12.4	11.9	12.0
	12.9	13.3	12.5	12.9	11.7	12.9	12.9	12.8	12.1	12.1
	13.0	13.4	13.1	12.9	12.3	13.3	13.1	12.9	13.0	12.1
	13.0	13.5	13.1	13.1	13.1	13.4	13.1	13.1	13.1	12.5
	13.2	13.6	13.2	13.3	13.2	14.3	13.3	13.2	13.2	12.6
	13.3	13.6	13.5	13.7	13.3	14.4	13.4	13.3	13.9	12.8
	13.8	14.0	13.9	14.4	13.9	14.5	14.4	13.5	14.8	13.2
Mean ± SD	12.9 ± 0.5	13.1 ± 0.6	12.8 ± 0.7	12.8 ± 0.9	11.2 ± 2.5	13.3 ± 0.8	12.9 ± 0.8	12.7 ± 0.6	12.7 ± 1.1	12.2 ± 0.5
Median	13.0	13.4	12.8	12.9	12.0	13.1	13.0	12.9	12.6	12.1

From McCarty (1987)

SD, standard deviation; U, units

[a] Sorted in ascending order.

males and females at 5 ppm, and cortical hypertrophy/hyperplasia was increased in males at 5 ppm (Table 29). Because there is no clear dose–response relationship, the finding is considered not treatment related. At 5 ppm, an increase in duodenum epithelial hyperplasia was observed in females, and an increase in renal necrotizing arteritis was seen in males. At 0.1 and 5 ppm in males, the incidence of lung adenocarcinoma was increased, but no dose–response relationship was evident for these high-background tumours, and therefore a treatment relationship is not likely. In males at 1 and 5 ppm, seven and four hepatocellular adenomas were found, respectively, without any other correlating histological liver findings. In male mice, an increase in lymphoreticular tumours was observed at 1 and 5 ppm (8 out of 49 and 11 out of 50, respectively). One

Table 29. Incidence of non-neoplastic findings in the chronic study in mice given diets containing cadusafos for 94/97 weeks

Finding	Incidence (no. of occurrences)									
	Males					Females				
	Dietary concentration (ppm)									
	0	0.1	0.5	1	5	0	0.1	0.5	1	5
Adrenals of animals examined	48	48	50	48	49	50	50	50	50	50
- Cortical atrophy	9	20*	13	16	19*	10	16	21	14	22*
- Cortical hypertrophy/hyperplasia	8	18*	10	15	23**	0	7	5	0	0
Duodenum of animals examined	49	26	34	23	48	50	50	50	50	50
- Epithelial hyperplasia	1	0	0	0	2	6	6	11	5	17**
Kidneys of animals examined	49	50	50	49	50	50	50	50	50	50
- Necrotizing arteritis	3	4	5	11*	12*	1	4	2	3	3

From McCarty (1987)
* $P \leq 0.05$; ** $P \leq 0.01$

Table 30. Incidence of neoplastic lesions in the chronic study in mice given diets containing cadusafos for 94/97 weeks

Lesion	Incidence (no. of occurrences)										
	Males					Females					Historical control group
	Dietary concentration (ppm)										
	0	0.1	0.5	1	5	0	0.1	0.5	1	5	
No. of animals examined	49	50	50	49	50	50	50	50	50	50	49
Lung adenoma	7	6	1	1	3	2	3	4	2	2	—
Lung adenocarcinoma	12	23	13	16	23	16	14	13	10	12	—
Total lung tumours	19	29*	14	17	26	18	17	17	12	14	—
Lymphoreticular tumours	6	5	5	8	11	24	23	29	15	24	7
Hepatocellular adenoma	1	1	2	7	4	0	1	0	1	0	—
Hepatocellular adenocarcinoma	0	0	0	0	1	1	0	0	0	0	—
Total hepatocellular tumours	1	1	2	7*	5	1	1	0	1	0	—

From McCarty (1987)
* $P \leq 0.05$

contemporary historical control group showed an incidence of 7 out of 49 (Table 30). External pathology experts assigned to review the original slides judged this finding to be spontaneous and probably not treatment related, because the incidence was mild and within the range one would expect from ageing mice. Furthermore, they concluded that there was no evidence for further neoplastic progression or decreased latency.

The NOAEL was 0.5 ppm, equal to 0.072 mg/kg bw per day, based on histological changes in the kidneys of male mice at 1 ppm, equal to 0.141 mg/kg bw per day. The NOAEL for cholinesterase activity inhibition was 1 ppm, equal to 0.141 mg/kg bw per day, based on plasma and erythrocyte cholinesterase inhibition in males and females and brain cholinesterase inhibition in males at 5 ppm, equal to 0.705 mg/kg bw per day in males and 1.008 mg/kg bw per day in females (McCarty, 1987).

Table 31. Clinical signs in rats given diets containing cadusafos for 100/104 weeks[a]

Dietary concentration (ppm)	Decreased locomotion		Lacrimation	
	Males	Females	Males	Females
0	68 (17)	107 (11)	1 (1)	29 (1)
0.1	133 (19)	76 (13)	0	7 (2)
0.5	114 (12)	70 (14)	2 (1)	0
1	144 (18)	81 (15)	0	0
5	49 (14)	363 (23)	17 (5)	4 (3)

From Weiner (1986)

[a] Numbers given are the number of observations made, with the number of animals affected in parentheses.

Rats

Groups of 60 male and 60 female 30-day-old Sprague-Dawley rats (Tac(SD)fBR) were fed diets containing 0, 0.1, 0.5, 1 or 5 ppm cadusafos (lot No. E2876-8; purity 93.42%) for 100 weeks (males) or 104 weeks (females). The concentrations of cadusafos in food were equal to doses of 0, 0.0044, 0.022, 0.045 and 0.222 mg/kg bw per day in males and 0, 0.0056, 0.028, 0.055 and 0.28 mg/kg bw per day in females.

Animals were observed regularly for appearance, behaviour and clinical signs, and body weight and food consumption were recorded weekly within the first 13 weeks and at 4-week intervals thereafter. Ophthalmology was assessed prior to study initiation and at study termination. Blood and urine samples for haematology, clinical chemistry and urinalysis, as well as for plasma and erythrocyte cholinesterase activity inhibition assays, were collected prior to the study, at 6, 12 and 18 months and at study termination. At 1 month, additional samples were collected for plasma and erythrocyte cholinesterase activity inhibition assays. At 12 months, 10 animals of each sex per group were killed for an interim examination. Both the interim kill animals and the animals sacrificed at study termination were subjected to a full necropsy, organ weights were recorded and histopathology was performed. The study complied with GLP.

The survival curves, food consumption, body weight development, clinical chemistry, haematology, urinalysis, organ weights, ophthalmological findings and histological non-neoplastic and neoplastic lesions were comparable among all groups. In females of the highest dose group, locomotion activity was decreased, on the basis of both the number of observations and the number of animals affected (Table 31). Additionally, slightly more males showed lacrimation in the high dose group when compared with all other groups. Whereas brain cholinesterase activity was not inhibited at 12 months or at study termination in any group, plasma and erythrocyte cholinesterase activities were inhibited, mostly statistically significantly, throughout the whole dosing period in males and females at 5 ppm (Tables 32 and 33).

The NOAEL was established at 1 ppm, equal to 0.045 mg/kg bw per day in males and 0.055 mg/kg bw per day in females, based on inhibition of erythrocyte cholinesterase activity and depressed locomotor activity in females at 5 ppm, equal to 0.222 mg/kg bw per day in males and 0.28 mg/kg bw per day in females (Weiner, 1986).

2.4 Genotoxicity

Cadusafos was not mutagenic in in vitro or in vivo tests (Table 34).

Table 32. Cholinesterase activity in plasma and erythrocytes in rats given diets containing cadusafos for 100/104 weeks

Dietary concentration (ppm)	Cholinesterase activity (% of control)									
	Males					Females				
	Time point (months)									
	1	6	12	18	Terminal	1	6	12	18	Terminal
Plasma										
0	100	100	100	100	100	100	100	100	100	100
0.1	94	98	94	102	144	81	103	102	102	113
0.5	104	100	75	110	165	97	96	122	97	110
1	100	92	79	86	138	103	98	113	91	95
5	82*	78**	80*	63**	94	48*	51**	61*	43**	48*
Erythrocytes										
0	100	100	100	100	100	100	100	100	100	100
0.1	106	98	110	104	94	101	93	107	94	103
0.5	104	96	109	108	97	102	94	105	99	97
1	103	101	110	105	95	99	93	107	98	92
5	83*	79**	98	77**	87**	69**	75**	91*	79**	83*

From Weiner (1986)
* $P \leq 0.05$; ** $P \leq 0.01$

Table 33. Brain cholinesterase activity at the interim kill and at study termination in rats given diets containing cadusafos for 100/104 weeks

Dietary concentration (ppm)	Cholinesterase activity (% of control)			
	Males		Females	
	Interim	Terminal	Interim	Terminal
0.1	105	103	97	99
0.5	105	102	99	99
1	98	102	98	97
5	98	101	101	100

From Weiner (1986)

Table 34. Results of genotoxicity studies with cadusafos

End-point	Test system	Concentration	Lot No.; purity (%)	Result	Reference
Reverse mutation (Ames)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0–1200 µg/plate (+S9) and 0–340 µg/plate (−S9)	E2876-8; 94.9	Negative	Haworth (1984)
	S. typhimurium TA98, TA100, TA102, TA1535, TA1537	0–5000 µg/plate (±S9)	PL03-0412; 91.3	Negative	Wagner (2005)
HGPRT mutation	CHO cells	0–140 µg/ml (+S9) and 0–95 µg/ml (−S9)	E2876-8; not specified	Negative −S9, not interpretable +S9[a]	Putman (1985)
		0–75 µg/ml (+S9) and 0–125 µg/ml (−S9)	E2876-8; not specified	Negative	Stankowski (1985)

Table 34 (contd)

End-point	Test system	Concentration	Lot No.; purity (%)	Result	Reference
Chromosomal aberration	CHO cells	0–75 µg/ml (±S9)	E2876-8; not specified	Negative[b]	Thilagar (1984a)
Unscheduled DNA synthesis	Rat primary hepatocytes	0–45 µg/ml	E2876-8; not specified	Negative	Thilagar (1984b)
Cell transformation	BALB/3T3 cells	0–90 µg/ml (+S9) and 0–70 µg/ml (−S9)	E2876-8; not specified	Equivocal[c]	Putman (1984)
Chromosomal aberration	Rat (Sprague-Dawley CD), single dose	68.3 mg/kg bw	PL 87-23; 91.9	Negative	Marshall (1989)

CHO, Chinese hamster ovary; S9, 9000 × *g* rat liver supernatant

[a] Without S9, a doubling of mutants at an intermediate concentration was observed; with S9, results are not interpretable owing to excessive toxicity.

[b] With S9 at the highest dose level, 4% of aberrant cells were observed, and 2% in control cells.

[c] In two assays with S9, the following transformation frequencies ($\times 10^{-4}$) were observed at concentrations of 0, 60, 70, 80 and 90 µg/ml: in the first assay, 0.15, 0.16, 0.52, 0.70* and 0.19, and in the second assay, 0.17, 0.18, 2.05**, 0.53 and 5.33** (* $P \leq 0.05$, ** $P \leq 0.01$); the relative (to control) cloning efficiencies were 100%, 91.5%, 87.2%, 80.9% and 80.9% in the first assay and 100%, 97.7%, 69.8%, 58.1% and 23.3% in the second assay.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 25 male and 25 female 7-week-old Sprague-Dawley (Tac(SD)fBR) rats were fed diets containing 0, 0.1, 0.5 or 5 ppm cadusafos (lot No. E2876-8; purity 93.42%). The average compound intake in the F_0 generation was 0, 0.0051, 0.026 and 0.262 mg/kg bw per day in males, 0, 0.0069, 0.034 and 0.340 mg/kg bw per day in non-pregnant females and 0, 0.0059, 0.029 and 0.297 mg/kg bw per day in females during gestation. After 8 weeks of exposure, the F_0 generation was allowed to mate and produce the F_{1a} and F_{1b} generations. F_{1a} pups were sacrificed upon weaning at lactation day 21, and F_{1b} pups were used to generate F_2.

Animals were observed regularly for appearance, behaviour and clinical signs, and body weight and food consumption were recorded during the premating phase, gestation phase and lactation phase. Ophthalmology was assessed prior to study initiation and at study termination. Blood samples of 10 males and 10 females per group for plasma and erythrocyte cholinesterase activity inhibition assays were collected 1 week before mating and 1 week after weaning. In these animals, cholinesterase activity of brain homogenates was also measured after terminal sacrifice. Pups were sexed and weighed at lactation days 4, 7, 14 and 21. A complete necropsy was performed on all adult animals and on 10 pups of all other groups. Additionally, organs were weighed and subjected to a histological examination. The study complied with GLP.

In F_0 and F_1, survival was not affected by treatment, and no treatment-related clinical signs were observed. Body weight development was not affected in F_0 at any dose in males or females. In F_1, males at 5 ppm had lower body weights when compared with control animals (Table 35). Lactating females in all dose groups had a slightly lower body weight than control animals, reaching statistical significance at 0.1 ppm at the F_{2a} and F_{2b} lactation period and at 5 ppm in the F_{2b} lactation period. As the effect is mild and no dose–response relationship is evident, it is of questionable toxicological relevance. With the exception of a slightly lower relative liver to brain weight in males at 5 ppm, no other treatment-related organ weight effects or histological changes were observed in F_0. In F_1, male animals at 5 ppm had statistically significantly lower absolute brain weights (−7%) and lower absolute liver weights (−13%).

Table 35. Body weights in F_1 parental rats given diets containing cadusafos in a study of reproductive toxicity

	Body weight (g ± SD)			
	Dietary concentration (ppm)			
	0	0.1	0.5	5
Males	615.6 ± 54.65	600.0 ± 50.69	597.0 ± 44.73	569.2 ± 48.47**
Females	356.0 ± 26.92	339.2 ± 29.87	346.4 ± 29.5	336.1 ± 22.48
Females at GD 20[a]	402.4 ± 33.96	390.4 ± 25.60	390.7 ± 38.46	395.6 ± 30.25
Females at LD 21[a]	336.0 ± 20.98	315.7 ± 23.67**	327.2 ± 20.67	324.2 ± 19.08
Females at GD 20[b]	438.7 ± 34.45	421.6 ± 27.87	408.5 ± 35.42**	421.8 ± 32.58
Females at LD 21[b]	365.8 ± 22.73	345.8 ± 22.09*	349.5 ± 28.55	343.7 ± 20.15*

From DeProspo (1987)
GD, gestation day; LD, lactation day; SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$
[a] Females at F_{2a} gestation.
[b] Females at F_{2b} gestation.

Table 36. Cholinesterase activity in plasma and erythrocytes in rats given diets containing cadusafos in a study of reproductive toxicity

Dietary concentration (ppm)	Cholinesterase activity (% of control)							
	Males				Females			
	F_0		F_1		F_0		F_1	
	Premating	Terminal	Premating	Terminal	Premating	Terminal	Premating	Terminal
Plasma								
0	100	100	100	100	100	100	100	100
0.1	92	96	100	97	105	125	78	88
0.5	98	96	98	95	111	102	71	75
5	84	82*	81	73**	55**	48**	43**	44**
Erythrocytes								
0	100	100	100	100	100	100	100	100
0.1	104	105	103	96	100	101	99	97
0.5	99	101	97	98	100	98	96	98
5	82**	89	80**	81**	82**	82**	75**	82**

From DeProspo (1987)
* $P \leq 0.05$; ** $P \leq 0.01$

No treatment-related histological changes were observed in any group of F_1. Plasma and erythrocyte cholinesterase activities were inhibited statistically significantly at 5 ppm in F_0 and F_1 males and females before mating and after weaning (Table 36). No effect on brain cholinesterase activity was observed at any dose level. Organ weights in progeny of all dose groups were not affected by treatment. The reproductive performance, live birth index and pup body weights were not affected by treatment in F_0. At 0.1 and 5 ppm, the survival index of pups at postnatal day (PND) 4 was statistically significantly lower than in controls (92% and 93% versus 97%). However, this finding is considered not to be treatment related, because it was not dose related and it was found only in F_{1a} and not in F_{1b}. In F_{2b}, 4.0% and 4.9% of stillborn pups were observed at 0.5 and 5 ppm, respectively, and 0.7% in controls (Table 37). Although the effect was statistically significant, it is considered incidental, because the concurrent control group had an unusually low incidence of stillborn pups. When compared with the control groups of F_{1a}, F_{1b} and F_{2a}, the incidences in the 0.5 and 5 ppm groups of F_{2b} are no longer significant.

Table 37. Stillborn pups in rats given diets containing cadusafos in a study of reproductive toxicity

	No. (%) of stillborn pups			
	Dietary concentration (ppm)			
	0	0.1	0.5	5
F_{1a} litter	13/265 (4.9)	8/282 (2.8)	18/260 (6.9)	8/252 (3.2)
F_{1b} litter	10/215 (4.7)	10/237 (4.2)	12/304 (3.9)	4/264 (1.5)
F_{2a} litter	21/278 (7.6)	17/287 (5.9)	21/255 (8.2)	16/295 (5.4)
F_{2b} litter	2/285 (0.7)	6/296 (2.0)	10/249 (4.0)**	13/265 (4.9)**

From DeProspo (1987)
** $P \leq 0.01$

The NOAEL for parental toxicity was established as 0.5 ppm, equal to 0.026 mg/kg bw per day, based on inhibition of plasma and erythrocyte cholinesterase activities at 5 ppm. The NOAEL for reproductive and developmental toxicity was established as 5 ppm, equal to 0.262 mg/kg bw per day, the highest dose tested (DeProspo, 1987).

(b) Developmental toxicity

Rats

Groups of 10 mated female Sprague-Dawley rats were administered cadusafos (lot No. E2876-8; purity 94.2%) in corn oil by gavage at 0, 0.2, 1, 5 or 20 mg/kg bw per day from gestation day (GD) 6 to GD 15. On GD 20, animals were sacrificed, and the number and distribution of implantation sites, early and late resorptions, live and dead fetuses and corpora lutea were recorded. The fetuses were weighed and sexed and investigated for gross external aberrations. No measurements of cholinesterase activity in plasma, erythrocytes or brain were performed. The study complied with GLP.

One high-dose dam died on each of study days 10 and 13, and all animals showed signs of intoxication from day 6 to day 20 as decreased locomotion, tremor, oral discharge, diarrhoea, abdominogenital staining, chromorhinorrhoea, recumbency and alopecia. The animal that died on day 10 had blood-tinged fluid in the thoracic and visceral cavities and clotted blood in the stomach and intestines. Mean food intake and mean body weight gain were reduced in the high dose group only. The numbers of corpora lutea, implantation sites or resorptions, litter size, number of fetuses, fetal body weights and sex ratios were not affected by treatment. No treatment-related gross external aberrations were observed. A slight decrease in mean live fetal body weight was observed at 20 mg/kg bw per day.

The maternal NOAEL and the developmental NOAEL were established as 5 mg/kg bw per day, based on deaths and clinical signs in dams at 20 mg/kg bw per day and decreased live fetal weights at 20 mg/kg bw per day (Freeman, 1984c).

Groups of 25 mated female Sprague-Dawley rats were administered cadusafos (lot No. E2876-8; purity 94.2%) by gavage in corn oil at 0, 2, 6 or 18 mg/kg bw per day from GD 6 to GD 15. On GD 20, animals were sacrificed, and the number and distribution of implantation sites, early and late resorptions, live and dead fetuses and corpora lutea were recorded. The fetuses were weighed and sexed and investigated for gross external, visceral and skeletal aberrations. No measurements of cholinesterase activity in plasma, erythrocytes or brain were performed. The study complied with GLP.

In the highest dose group, maternal body weights were decreased at days 15 and 20 by 9% and 7%, respectively. In this group, the mean feed intake during gestation was also decreased by 8–16%. One animal of the 6 mg/kg bw per day group showed signs of intoxication from day 14 to day 18 as decreased locomotion, tremor, oral discharge, diarrhoea, abdominogenital staining and

Table 38. Skeletal findings in fetuses in a study of developmental toxicity in rats given cadusafos by gavage

Skeletal findings	Incidence of skeletal findings			
	Dose (mg/kg bw per day)			
	0	2	6	18
Sternebrae 2 absent				
Litter	2/23	1/25	1/25	4/25
Fetuses	2/140	1/150	1/149	5/162
Sternebrae 5 absent				
Litter	7/23	8/25	5/25	19/25**
Fetuses	8/140	10/150	6/149	55/162*
Sternebrae 5 split				
Litter	0/23	1/25	0/25	3/25
Fetuses	0/140	1/150	0/149	3/162
Xiphoid absent				
Litter	1/23	4/25	8/25	13/25***
Fetuses	1/140	5/150	9/149	30/162*
Thoracic vertebrae centra split				
Litter	3/23	6/25	8/25	8/25
Fetuses	6/140	6/150	9/149	14/162
Caudal vertebrae absent				
Litter	14/23	17/25	16/25	21/25
Fetuses	34/140	41/150	29/149	87/162*
Metacarpal(s) absent				
Litter	3/23	1/25	4/25	9/25
Fetuses	3/140	1/150	6/149	17/161****
Supraoccipital partially ossified				
Litter	2/23	3/25	6/25	7/25
Fetuses	2/140	3/149	8/149	11/161****

From Freeman (1985b)
* $P < 0.01$; ** $P = 0.002$; *** $P < 0.001$; **** $P < 0.05$

chromorhinorrhoea. All animals of the high dose group showed severe signs of intoxication starting on GD 7. Litter data were not affected by treatment in any group. Body weights of high-dose male pups were reduced by 8%, and those of high-dose female pups by 6%. One 18 mg/kg bw per day fetus had microphthalmia, another one an umbilical hernia. At 6 mg/kg bw per day, one fetus had no tail, a blind colon and atresia of the genital papillae, and another one, anophthalmia. At 18 mg/kg bw per day, more fetuses with absent sternebrae were found, and at 6 and 18 mg/kg bw per day, there were more fetuses with absent xiphoid (Table 38). There were also statistically significant increases in partially ossified supraoccipital bone, sternebrae and absent metacarpals at 18 mg/kg bw per day. Although not statistically significant, a dose-related increase in litter and fetus incidence of dilated ureters was found (Table 39).

The NOAEL for maternal toxicity was established as 2 mg/kg bw per day, based on clinical signs in dams at 6 mg/kg bw per day. The NOAEL for developmental toxicity was 2 mg/kg bw per day, based on absent xiphoids in fetuses at 6 mg/kg bw per day (Freeman, 1985b).

Table 39. Visceral findings in fetuses in a study of developmental toxicity in rats given cadusafos by gavage

Visceral finding	Incidence of visceral findings			
	Dose (mg/kg bw per day)			
	0	2	6	18
Dilated ureter				
Litter	8/23	10/25	9/25	12/25
Fetuses	14/137	17/145	19/153	26/166

From Freeman (1985b)

Table 40. Clinical signs in a range-finding study of developmental toxicity in rabbits given cadusafos by gavage[a]

Clinical signs	Dose (mg/kg bw per day)				
	0	0.2	1	5	20
Body weight gain, days 7–19 (kg ± SD)	0.12 ± 0.208	−0.16 ± 0.204	−0.15 ± 0.203	−0.51 ± 0.332	—
Mortality	0	0	2 (8, 20)	3 (8, 16, 26)	8 (7, 11, 11, 13, 14, 16, 16)[b]
Abortion	1 (29)	0	0	0	0
Early delivery	0	1 (29)	1 (29)	1[c]	—

From Freeman (1985b)

—, not relevant due to high mortality; SD, standard deviation

[a] Day of observation in parentheses.

[b] Day of death of animal No. B2570F was not reported.

[c] Day of delivery not clear.

Rabbits

In a range-finding study, groups of eight mated female New Zealand White rabbits were administered cadusafos (lot No. E2876-8; purity 94.9%) by gavage in corn oil at 0, 0.2, 1, 5 or 20 mg/kg bw per day from GD 7 to GD 19. On GD 29, animals were sacrificed, and the number and distribution of implantation sites, early and late resorptions, live and dead fetuses and corpora lutea were recorded. The fetuses were weighed and sexed and investigated for gross external, visceral and skeletal aberrations. Cholinesterase activities in plasma and erythrocytes of five animals per group were measured on study days 6 (pretreatment), 14 and 29, and brain cholinesterase activity was measured on terminally sacrificed animals. The study complied with GLP.

At 1 mg/kg bw per day and above, an increase in mortality was observed, and the surviving animals showed lower body weight gain (Table 40). There was no increase in abortions or early delivery seen at 0.2, 1 or 5 mg/kg bw per day; this parameter could not be evaluated at the high dose because of high mortality. Clinical signs at 1, 5 or 20 mg/kg bw per day included ataxia, cyanosis, loss of muscle control, diarrhoea, dyspnoea, oral discharge, tremors, abdominogenital staining and decreased locomotion. Erythrocyte and plasma cholinesterase activities were markedly decreased at all dose levels by day 14 (after 7 days of treatment) and day 29 (10 days after dosing cessation) (Table 41). The significance of the brain cholinesterase activity measured at terminal sacrifice (Table 42) is questionable, as at this time point, the last dosing had occurred 10 days previously.

Table 41. Erythrocyte and plasma cholinesterase activities in a range-finding study of developmental toxicity in rabbits given cadusafos by gavage

	Cholinesterase activities (%)[a]				
	Dose (mg/kg bw per day)				
	0	0.2	1	5	20
Erythrocytes					
Day 14	96	60	31	25	—
Day 29	92	68	43	34	—
Plasma					
Day 14	85	57	50	19	—
Day 29	60	50	51	51	—

From Freeman (1985b)

—, not relevant due to high mortality

[a] Mean of individual activities at days 14 and 29 compared with pretreatment (day 6).

Table 42. Mean terminal brain cholinesterase activity in a range-finding study of developmental toxicity in rabbits given cadusafos by gavage

	Dose (mg/kg bw per day)				
	0	0.2	1	5	20
No. of animals	5	5	4	2	0
Brain cholinesterase activity (U/g) (± SD)	16.6 ± 3.3	15.9 ± 1.0	14.8 ± 1.9	12.3 ± 2.1	—

From Freeman (1985b)

—, not relevant due to high mortality; SD, standard deviation; U, units

A NOAEL could not be determined, because at the lowest dose level of 0.2 mg/kg bw per day, marked erythrocyte cholinesterase inhibition and body weight loss were observed (Freeman, 1984b).

Groups of 20 mated female New Zealand White rabbits were administered cadusafos (lot No. E2876-8; purity 94.2%) by gavage in corn oil at 0, 0.1, 0.3 or 0.9 mg/kg bw per day from GD 7 to GD 19. On GD 29, animals were sacrificed, and the number and distribution of implantation sites, early and late resorptions, live and dead fetuses and corpora lutea were recorded. The fetuses were weighed and sexed and investigated for gross external, visceral and skeletal aberrations. No measurements of cholinesterase activity in plasma, erythrocytes or brain were performed. The study complied with GLP.

One control rabbit died at day 29 for unknown reasons, and another one was sacrificed after preterm delivery on day 29 (Table 43). At 0.1 mg/kg bw per day, one animal aborted on day 22 and was sacrificed, and another one had preterm delivery on day 28 and was sacrificed as well. At 0.3 mg/kg bw per day, one animal died on day 15. At 0.9 mg/kg bw per day, two animals aborted on day 27, one delivered on day 28 and two animals died, one on day 20 and the other one on day 23. The company stated that the most likely cause of death of animals of the 0.3 and 0.9 mg/kg bw per day groups was convulsive seizures. Additionally, an increased incidence of several other clinical signs of intoxication was observed at 0.3 mg/kg bw per day and above. There were no effects on body weight development or food consumption. No treatment-related effects were observed on fertility, number of corpora lutea, number of implantation sites, litter size, sex ratio, viability, fetal body weight or skeletal or visceral development. A slight and statistically not significant increase in early resorptions was observed at 0.3 mg/kg bw per day and above (Table 44), but the total number of resorptions

Table 43. Clinical signs in a study of developmental toxicity in rabbits given cadusafos by gavage

Clinical signs	Incidence (no. of occurrences)[a]			
	Dose (mg/kg bw per day)			
	0	0.1	0.3	0.9
Rales	1 (19)	0	2 (15, 16)	4 (18, 16, 16, 15)
Diarrhoea	0	1 (9)	1 (29)	4 (8, 15, 21, 22)
Prostrate	0	0	1 (15)	1 (23)
Dyspnoea	0	0	1 (15)	1 (19)
Hypersensitivity	0	0	0	2 (9, 9)
Ataxia	0	0	0	1 (19)
Staggered gait	0	0	0	1 (19)
Loss of muscle control	0	0	0	1 (23)
Found dead	1 (29)	0	1 (15)	2 (20, 23)
Preterm delivery	1 (29)	1 (28)	0	1 (28)
Abortion	0	1 (22)	0	2 (27, 27)

From Freeman (1985a)

[a] Day of observation in parentheses.

Table 44. Number of resorptions in a study of developmental toxicity in rabbits given cadusafos by gavage

	Number of resorptions[a]				
	Dose (mg/kg bw per day)				Historical control data[b]
	0	0.1	0.3	0.9	
Early	8 (5)	7 (4.1)	15 (8.8)	15 (11.2)	3 (1.8)
Late	8 (5.0)	9 (5.3)	3 (1.8)	4 (1.8)	13 (7.9)
Total	16 (9.9)	16 (9.4)	18 (10.5)	19 (14.2)	16 (9.7)

From Freeman (1985a)

[a] % of implantations in parentheses.

[b] One study from 1984.

did not change significantly. Comparing the ratio of early and late resorptions of the current control group with the ratio in the historical control group, high variability is evident. Therefore, the increase in early resorptions was considered to be incidental.

The NOAEL for maternal toxicity was established as 0.1 mg/kg bw per day, based on clinical signs at 0.3 mg/kg bw per day. The NOAEL for developmental toxicity was established at 0.9 mg/kg bw per day, the highest dose tested (Freeman, 1985a).

2.6 Special studies

(a) Neurotoxicity

Rats

In an acute neurotoxicity study, groups of 20 male and 20 female Sprague-Dawley CD rats were administered a single dose of cadusafos (lot No. PL97-1570; purity 91.7%) at 0, 0.02, 25 or 40 mg/kg bw by oral gavage. For part I of the study, 10 animals per group were used for standard neurotoxicity tests, including recording of clinical signs, body weight development and functional

Table 45. Cholinesterase activity in plasma, erythrocytes and brain in an acute neurotoxicity study in rats given cadusafos by gavage

Dose (mg/kg bw per day)	Cholinesterase activity (% of control)			
	Day 0		Day 14	
	Males	Females	Males	Females
Plasma				
0	100	100	100	100
0.02	89	97	110	186
25	5	2	107	153
40	4*	1*	107	142
Erythrocytes				
0	100	100	100	100
0.02	119	111	96	113
25	27	34	96	148
40	38*	42*	94	124*
Brain				
0	100	100	100	100
0.02	91	82	92	100
25	94	76	100	142
40	86	52	108	142*

From Watt (2001a)
* $P \leq 0.05$

observational battery (FOB) and motor activity testing; at terminal sacrifice, half of these animals were investigated for gross lesions, and the other half underwent histological examination of nervous tissues. For part II of the study, the other half of the initial 20 animals were used for plasma, erythrocyte and brain cholinesterase activity measurements; 5 animals were analysed at day 0 directly after dosing, and the remaining 5 animals at day 14. The study complied with GLP.

Two females of the 40 mg/kg bw group died on treatment days 2 and 3, respectively. Treatment-related clinical signs were noted in animals receiving 25 or 40 mg/kg bw. These signs included diarrhoea, abdominogenital staining, oral discharge, decreased locomotion, decreased faeces, lacrimation, haematuria, tremors and unthriftiness. These signs resolved within 5 days. At 25 mg/kg bw, females were soiled by body fluids on day 0. Both sexes had reduced motor activity at 25 and 40 mg/kg bw. At 40 mg/kg bw, males were soiled by body fluids and displayed reduced motor activity in the open field. Females at this dose level were soiled by body fluids on day 0, were limp when handled and showed abnormal posture, tremors, staggered gait, splayed hind limbs and reduced motor activity in the open field, reduced hind limb grip strength and a significant increase in tail flicking latency. At day 7 and day 14, males and females at all dose levels did not show any FOB effects. At study termination, no gross lesions or microscopic changes in nervous tissues were observed in males or females at any dose level.

In males and females at 40 mg/kg bw, plasma and erythrocyte cholinesterase activities were inhibited statistically significantly on day 0, and full activity was observed at day 14 (Table 45). At 25 mg/kg bw, plasma and erythrocyte cholinesterase activities were also inhibited, but not statistically significantly. Brain cholinesterase activity was not statistically significantly inhibited in any dose group, but individual data show more animals with low brain cholinesterase activity at 25 and 40 mg/kg bw (Table 46).

Table 46. Individual brain cholinesterase activity at day 0 in an acute neurotoxicity study in rats given cadusafos by gavage[a]

	Brain cholinesterase activity (U/g)							
	Females				Males			
	Dose (mg/kg bw per day)							
	0	0.02	25	40	0	0.02	25	40
	2.6	1.9	1.6	0.8	3.0	2.4	1.6	2.7
	3.0	2.1	1.7	1.0	3.1	2.7	2.0	3.2
	3.3	3.0	2.8	1.2	3.4	3.2	3.9	3.2
	3.6	3.2	3.0	1.3	3.7	3.9	4.1	3.5
	4.2	3.2	3.2	4.0	4.1	3.9	5.0	3.6
Mean ± SD	3.3 ± 0.6	2.7 ± 0.6	2.5 ± 0.8	1.7 ± 1.3	3.5 ± 0.5	3.2 ± 0.7	3.3 ± 1.5	3.2 ± 0.4
Median	3.3	3.0	2.8	1.2	3.4	3.2	3.9	3.2

From Watt (2001a)

SD, standard deviation; U, units

[a] Sorted in ascending order.

The NOAEL in this study was established at 0.02 mg/kg bw, based on inhibition of erythrocyte and brain cholinesterase activities, FOB effects and clinical signs at 25 mg/kg bw (Watt, 2001a).

Groups of 15 male and 15 female Sprague-Dawley CD rats were fed diets containing cadusafos (lot No. PL97-1570; purity 91.7%) at concentrations of 0, 0.1, 0.5 or 300 ppm (equal to 0, 0.006, 0.031 and 20.0 mg/kg bw per day in males and 0, 0.007, 0.037 and 23.1 mg/kg bw per day in females) for 13 weeks. For part I of the study, 10 animals per group were used for standard neurotoxicity tests, including recording of clinical signs, body weight development and FOB and motor activity testing; at terminal sacrifice, half of these animals were investigated for gross lesions, and the other half underwent histological examination of nervous tissues. For part II of the study, the remaining five animals of each sex per group were used for plasma, erythrocyte and brain cholinesterase activity measurements at study termination. The study complied with GLP.

All animals survived until study termination. Females at 300 ppm showed increased hypersensitivity and decreased faeces. Males at 300 ppm had a statistically significant lower body weight (−19%) at the end of the study (Table 47). The 300 ppm males displayed a reduction in the landing foot splay parameter at weeks 4 and 13, and forelimb grip strength was reduced. No other FOB effects were observed, and motor activity was not affected in any dose group. Nor were any treatment-related gross lesions or histological changes in the nervous system seen. In the 300 ppm groups at study termination, plasma and brain cholinesterase activities were reduced statistically significantly in both sexes, and erythrocyte cholinesterase activity was statistically significantly reduced in males (Table 48).

The NOAEL was established as 0.5 ppm, equal to 0.031 mg/kg bw per day, based on clinical signs, reduced body weights and reduced plasma, erythrocyte and brain cholinesterase activities at 300 ppm, equal to 20.1 mg/kg bw per day in males and 23.1 mg/kg bw per day in females (Watt, 2001b).

In a study to titrate plasma, erythrocyte and brain cholinesterase activities to identify the dose range for longer-term studies, groups of 15 male and 15 female rats (strain not specified) were fed

Table 47. Terminal body weights and terminal body weight gains in a neurotoxicity study in rats given diets containing cadusafos for 13 weeks

Dose (mg/kg bw per day)	Body weight (% of control)	Body weight gain (% of control)
Males		
0	100	100
0.1	96	93
0.5	94	91
300	81*	71*
Females		
0	100	100
0.1	101	101
0.5	100	99
300	106	116

From Watt (2001b)
* $P \leq 0.05$

Table 48. Terminal cholinesterase activity in a neurotoxicity study in rats given diets containing cadusafos for 13 weeks

Dose (mg/kg bw per day)	Cholinesterase activity (% of control)	
	Males	Females
Plasma		
0	100	100
0.1	112	116
0.5	117	83
300	0*	0*
Erythrocytes		
0	100	100
0.1	96	106
0.5	116	126
300	21*	25
Brain		
0	100	100
0.1	88	112
0.5	120	94
300	19*	16*

From Watt (2001b)
* $P \leq 0.05$

diets containing 0, 1, 2.5, 5, 10, 25 or 50 ppm cadusafos (lot No. E2876-8; purity 94.2%) for up to 4 weeks. The mean calculated compound intake was 0, 0.09, 0.23, 0.47, 0.94, 2.29 and 4.67 mg/kg bw per day in males and 0, 0.11, 0.24, 0.47, 0.99, 2.50 and 4.97 mg/kg bw per day in females, respectively. Body weight development and food intake were recorded. On days 0 (pretest), 3, 7, 14 and 28, blood samples were collected for plasma and erythrocyte cholinesterase activity measurements; at the same time points (except at 14 days), five animals were sacrificed for brain cholinesterase activity measurement. Individual cholinesterase activities were not reported. The study complied with GLP.

No animals died before study termination. There was no effect on body weight development or food consumption. Plasma cholinesterase activity in males was depressed at 10 ppm and above, and equivocal inhibition was observed at 5 ppm. In females, plasma cholinesterase activity was depressed at 2.5 ppm and above. Erythrocyte cholinesterase activity was low by day 28 at 5 ppm in both sexes and by day 14 onwards at 10 ppm in both sexes. Brain cholinesterase activity was inhibited in males at 10 ppm at day 28 and thereafter and in the 50 ppm group also at day 7. In females, brain cholinesterase activity was inhibited at 50 ppm on days 7 and 28 (Geiger, 1986).

Hens

To determine the LD_{50} in hens, groups of 10 hens were administered a single oral dose of 0, 2, 4, 8, 16 or 32 mg/kg bw of cadusafos (lot No. E2876-8) and observed for 14 days for body weight development, clinical signs and mortalities.

To study delayed neurotoxicity, four groups with 10 hens each were orally administered a single dose of cadusafos (lot No. E2876-8) at 8 mg/kg bw. A positive control group was administered a single oral dose of tri-*ortho*-cresyl phosphate (TOCP) at 500 mg/kg bw. Hens dosed with cadusafos were protected with atropine at 10 mg/kg bw immediately before dosing. The procedure was repeated after 21 days, followed by a further 21-day observation period; the animals were then sacrificed. Animals were observed for body weight development, food consumption, clinical signs, ataxia and mortalities; at final sacrifice, brain (medulla/pons, cerebellar and cerebral cortex), spinal cord (cervical, thoracic and lumbar-sacral regions) and peripheral nerves (proximal and distal sciatic nerve and tibial nerve) were examined histologically.

The LD_{50} was calculated as 7.7 mg/kg bw. The lowest dose causing mortality in one animal within 24 h was 4 mg/kg bw. At the lowest dose of 2 mg/kg bw and above, animals were subdued for at least 2 days.

In the delayed neurotoxicity study, 15 out of 40 birds died within 6 days, and 1 bird died after redosing. Shortly after dosing within 3 days, cadusafos-treated animals showed body weight loss; thereafter, body weight gain was normal. The surviving animals showed treatment-related clinical signs of unsteadiness, leg stiffness and weakness by the end of day 1. Surviving animals recovered by days 4–6. To a slightly lesser degree, similar signs were observed after redosing. Histologically, only 1 out of 10 dosed animals showed an axonal degeneration in the spinal cord that could be treatment related based on its severity. No histological findings of similar severity were made in the concurrent control group. However, the study director noted that findings of similar severity were occasionally made in other control hens. Therefore, the finding is inconclusively treatment related (Roberts, 1984).

3. Observations in humans

No reports on health effects in personnel exposed to cadusafos were submitted.

Comments

Biochemical aspects

Studies in male and female rats given [butyl-^{14}C]cadusafos at a dose of 1 mg/kg bw showed that the radiolabel was absorbed (highest blood concentrations being reached at about 4–8 h) and rapidly excreted (>80% of the administered dose within 24 h). Of the recovered radiolabel, 70–80% was excreted in the urine, 4–14% in the faeces and 12–18% as carbon dioxide. The results of a study with intravenous application of radiolabelled cadusafos suggested that approximately 5% of faecal excretion is attributable to biliary excretion. Oral absorption is therefore estimated to be close to 100% in

males and greater than 90% in females. Cadusafos was widely distributed among the organs, a peak of 1.2% of the administered dose being found in the body at 7 days after dosing. Highest concentrations were observed in the liver, fat, kidney and lungs. There was no evidence for accumulation of cadusafos in the body. Cadusafos is extensively metabolized in rats. Metabolism starts by cleavage of one of the thiobutyl groups to give butyl mercaptan and *O*-ethyl-*S*-(2-butyl) phosphorothioic acid, which can then be cleaved to *S*-(2-butyl) phosphorothioic acid or *O*-ethyl phosphorothioic acid. Butyl mercaptan is biotransformed to methyl *sec*-butyl sulfide, sulfoxide and sulfone and finally to hydroxysulfones. Alternatively, butyl mercaptan can be oxidized to butyl sulfonic acid, then to ethyl and methyl sulfonic acid. The results suggested that there are no significant differences between males and females in the toxicokinetic parameters and the metabolic profile observed with cadusafos at a dose of 1 mg/kg bw.

Toxicological data

Cadusafos was of high to moderate toxicity by the oral route, with an LD_{50} of 30–131 mg/kg bw in rats and 68–82 mg/kg bw in mice. By the dermal route, the LD_{50} was 12–42 mg/kg bw in rabbits. By inhalation, the LC_{50} was 0.04 mg/l air in rats. In rabbits, cadusafos was not irritating to the eye or the skin. In a Buehler test, no evidence for delayed-contact hypersensitivity was observed.

In studies with repeated doses, the main effect was the inhibition of cholinesterase activity in plasma, erythrocytes and brains of treated animals and related clinical and behavioural signs of intoxication.

In a 4-week feeding study in mice, the only effect was the inhibition of erythrocyte cholinesterase activity at 10 ppm and of brain cholinesterase activity at 33 ppm. The NOAEL was 3 ppm, equal to 0.83 mg/kg bw per day, on the basis of inhibition of erythrocyte cholinesterase activity at 10 ppm.

In a 4-week feeding study in rats, a NOAEL could not be identified because marked inhibition of cholinesterase activity and concomitant clinical signs were observed at the lowest dietary concentration tested, 50 ppm, equal to 4.7 mg/kg bw per day. In a 13-week feeding study in rats, very high mortality was observed at 800 ppm, and one female died at the next lower dietary concentration of 5 ppm. At 800 ppm, typical clinical signs of cholinesterase inhibition were identified. Inhibition of erythrocyte cholinesterase activity was seen in males and females at 5 ppm towards the end of the study and in males and females at 800 ppm at all time points. Brain cholinesterase activity was inhibited in females at 5 and 800 ppm and in males at 800 ppm. The NOAEL for rats was 1 ppm, equal to 0.067 mg/kg bw per day, on the basis of reduced erythrocyte and brain cholinesterase activity at 5 ppm.

In a 2-week study in dogs given capsules containing cadusafos, no treatment-related effects on erythrocyte and brain cholinesterase activity were observed up to 1 mg/kg bw per day, the highest dose tested. In a 13-week study in dogs given capsules, no effects on erythrocyte or brain cholinesterase activity were observed up to 0.09 mg/kg bw per day, the highest dose tested. The only effect was a decrease in mean testes weights at 0.03 mg/kg bw per day and above. Therefore, the NOAEL was 0.01 mg/kg bw per day. In a second 13-week study in dogs given a newer batch of cadusafos, the effect on testes weights was no longer observed up to 0.1 mg/kg bw per day, the highest dose tested. In a 1-year study in dogs fed capsules, no treatment-related clinical effects were observed and erythrocyte and brain cholinesterase activities were not inhibited at up to 0.02 mg/kg bw per day, the highest dose tested. The only finding was inhibition of plasma cholinesterase activity in females at 0.005 and 0.02 mg/kg bw per day. The Meeting considered that this effect was not toxicologically relevant, and the NOAEL was thus 0.02 mg/kg bw per day, the highest dose tested. The overall NOAEL for 13-week and 1-year studies in dogs was 0.09 mg/kg bw per day on the basis of the absence of any toxicologically relevant effects at the highest dose tested in the 13-week study.

Cadusafos was tested for genotoxicity in an adequate range of studies. In the submitted studies, there was no evidence for genotoxicity in vitro or in vivo.

The Meeting concluded that cadusafos was unlikely to be genotoxic.

In a 94/97-week feeding study in mice, plasma and erythrocyte cholinesterase activities were reduced in males and females and brain cholinesterase activity was reduced in males at the highest dietary concentration of 5 ppm, equal to 0.705 mg/kg bw per day. The incidences of non-neoplastic lesions such as cortical atrophy and hypertrophy/hyperplasia in the adrenals were increased in rats at 5 ppm when compared with controls, but there was no consistent dose–response relationship. Duodenal epithelial hyperplasia was increased in females at 5 ppm, and necrotizing arteritis of the kidneys was increased in males at 1 and 5 ppm. Non-dose-related increases in the incidences of lung and liver tumours in males were not considered to be treatment related. In males, an increase in the incidence of lymphoreticular tumours was observed (8 out of 49 and 11 out of 50 at 1 and 5 ppm, respectively, versus 6 out of 49 in controls) that was also greater than the incidence observed in one contemporary historical control group. As the increase was not statistically significant and as lymphoreticular tumours are common in ageing mice, the effect was not considered to be treatment related. The NOAEL was 0.5 ppm, equal to 0.072 mg/kg bw per day, on the basis of histological changes in the kidneys of male mice at 1 ppm. The Meeting concluded that cadusafos is not carcinogenic in mice.

In a 100/104-week feeding study in rats, females receiving the highest dietary concentration of 5 ppm showed decreased locomotion activity. Additionally, slightly more males showed lacrimation at this dietary concentration than did all other groups. Although brain cholinesterase activity was not inhibited at 12 months or at study termination in any group, plasma and erythrocyte cholinesterase activities were inhibited (mostly statistically significantly) throughout the whole dosing period in males and females at 5 ppm. No increase in the frequency of any non-neoplastic or neoplastic changes was observed. The NOAEL was 1 ppm, equal to 0.045 mg/kg bw per day, on the basis of inhibition of erythrocyte cholinesterase activity and depressed locomotor activity at 5 ppm. The Meeting concluded that cadusafos is not carcinogenic in rats.

In the absence of genotoxic and carcinogenic potential, the Meeting concluded that cadusafos is unlikely to pose a carcinogenic risk to humans.

The reproductive toxicity of cadusafos has been investigated in a two-generation study in rats. No treatment-related clinical signs were observed in any parental group. A slight and not dose-related decrease in the body weights of lactating F_1 females was observed at all doses. The Meeting considered this effect to be of questionable toxicological relevance. In F_1 males, a mild decrease in absolute liver and brain weights was observed without any histological correlates at 5 ppm, equal to 0.262 mg/kg bw per day. At 5 ppm, male and female F_0 and F_1 animals had statistically significantly lowered plasma and erythrocyte cholinesterase activities in the premating phase and at weaning. Reproductive performance, litter data and postnatal development were not affected by treatment. The NOAEL for parental toxicity was 0.5 ppm, equal to 0.026 mg/kg bw per day, on the basis of inhibition of erythrocyte cholinesterase activity at 5 ppm. The NOAEL for reproductive and developmental toxicity was 5 ppm, the highest dose tested.

The developmental toxicity of cadusafos has been investigated in rats and rabbits. In the study in rats, maternal body weights and food intake were decreased at the highest dose of 18 mg/kg bw per day. One rat in the group at 6 mg/kg bw per day and all rats at the highest dose showed severe signs of intoxication starting on day 7 of gestation. Litter data were not affected by treatment in any group. Body weights of male and female pups at the highest dose were reduced by 8% and 6%, respectively. The incidence of fetuses with absent sternebrae and partially ossified supraoccipital bone, sternebrae and absent metacarpals was increased at 18 mg/kg bw per day, and there were more fetuses with absent xiphoid at 6 and 18 mg/kg bw per day. A non-statistically significant increase in the incidence of dilated ureters in litters and fetuses was found at 18 mg/kg bw per day. The NOAEL for maternal toxicity was 2 mg/kg bw per day on the basis of clinical signs in dams at 6 mg/kg bw per day. The

NOAEL for developmental toxicity was 2 mg/kg bw per day on the basis of absent xiphoids in fetuses at 6 mg/kg bw per day.

In a range-finding study of developmental toxicity in rabbits, an increase in mortality (one death at study day 8 and another one at day 20) was observed at 1 mg/kg bw per day and above, and the surviving rabbits showed lower body weight gain compared with the controls. In the main study in rabbits, one rabbit died on day 15 at 0.3 mg/kg bw per day, two rabbits at 0.9 mg/kg bw per day aborted on day 27, one rabbit delivered on day 28 and two rabbits died (one on day 20 and the other on day 23). Additionally, increased incidences of several other clinical signs of neurotoxicity induced by cholinesterase inhibition were observed at doses of 0.3 mg/kg bw per day and above, starting on day 15. At a dose of 0.2 mg/kg bw per day, there was marked inhibition of erythrocyte cholinesterase activity in the range-finding study. At 0.3 and 0.9 mg/kg bw per day, the frequency of early resorptions was increased, whereas the frequency of late resorptions was decreased. The Meeting did not consider this finding to be treatment related, because the total number of resorptions was only minimally increased and because the ratio of early to late resorptions is highly variable. No treatment-related effects on fertility, number of corpora lutea, number of implantation sites, litter size, sex ratio, viability, fetal body weight, or skeletal or visceral development were observed. The NOAEL for maternal toxicity was 0.1 mg/kg bw per day on the basis of clinical signs at 0.3 mg/kg bw per day. The NOAEL for developmental toxicity was 0.9 mg/kg bw per day, the highest dose tested. The Meeting concluded that cadusafos was not teratogenic at doses that were not toxic to dams.

In a study of delayed neurotoxicity in hens, 1 out of 10 hens given cadusafos at a dose of 8 mg/kg bw (a potentially lethal dose) showed axonal degeneration in the spinal cord, but not in the peripheral nervous system. In view of the fact that clinical signs of delayed neuropathy were not observed and that axonal lesions in the spinal cord were observed occasionally in hens in the control group, the Meeting concluded that cadusafos is unlikely to cause delayed neuropathy at lethal doses.

In a study of acute neurotoxicity in rats, two females at 40 mg/kg bw died on treatment days 2 and 3, respectively. Treatment-related clinical signs were noted in rats at 25 and 40 mg/kg bw. These signs resolved within 5 days. Females at 40 mg/kg bw were soiled by body fluids on day 0, were limp when handled and showed abnormal posture, tremors, staggered gait, splayed hind limbs and reduced motor activity in the open field, reduced hind limb grip strength and a significant increase in tail-flicking latency. At days 7 and 14, no FOB effects were observed in any group. At study termination, no gross lesions or microscopic changes in nervous tissues were observed. At 25 and 40 mg/kg bw, plasma and erythrocyte cholinesterase activities were inhibited. Brain cholinesterase activity was not statistically significantly inhibited at any dose, but individual data showed an increase in the incidence of rats with low brain cholinesterase activity at 25 and 40 mg/kg bw. The NOAEL was 0.02 mg/kg bw on the basis of inhibition of erythrocyte and brain cholinesterase activities, FOB effects and clinical signs at 25 mg/kg bw. The Meeting noted the large dose spacing in the study of acute neurotoxicity. In a 13-week feeding study of neurotoxicity in rats, females at 300 ppm showed increased hypersensitivity; males at 300 ppm displayed a reduction in the landing foot splay parameter, and forelimb grip strength was reduced. No other FOB effects were observed, motor activity was not affected at any dose and no treatment-related gross lesions or histological changes in the nervous system were seen. In the groups at 300 ppm at study termination, plasma and brain cholinesterase activities were reduced statistically significantly in males and females, and erythrocyte cholinesterase activity was statistically significantly reduced in males. The NOAEL was 0.5 ppm, equal to 0.031 mg/kg bw per day, on the basis of clinical signs, reduced body weights and reduced erythrocyte and brain cholinesterase activities at 300 ppm. The Meeting considered that cadusafos is neurotoxic.

No reports on health effects in personnel exposed to cadusafos were submitted.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.0005 mg/kg bw based on a NOAEL of 1 ppm, equal to 0.045 mg/kg bw per day, identified on the basis of inhibition of erythrocyte cholinesterase activity at 5 ppm, equal to 0.222 mg/kg bw per day, in the long-term study in rats. A safety factor of 100 was applied.

The Meeting established an acute reference dose (ARfD) of 0.001 mg/kg bw based on a NOAEL of 0.1 mg/kg bw per day identified on the basis of clinical effects in dams at 0.3 mg/kg bw per day in the study of developmental toxicity in rabbits. A safety factor of 100 was applied. The large dose spacing between the LOAEL and the NOAEL in the study of acute neurotoxicity made this study unsuitable for the derivation of an ARfD. The Meeting also noted that the ARfD established might be conservative because it was derived using clinical signs that occurred only after administration of several doses.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	0.5 ppm, equal to 0.072 mg/kg bw per day	1 ppm, equal to 0.141 mg/kg bw per day
		Carcinogenicity	5 ppm, equal to 0.705 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	1 ppm, equal to 0.045 mg/kg bw per day	5 ppm, equal to 0.222 mg/kg bw per day
		Carcinogenicity	5 ppm, equal to 0.222 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	5 ppm, equal to 0.262 mg/kg bw per day[b]	—
		Parental toxicity	0.5 ppm, equal to 0.0262 mg/kg bw per day	5 ppm, equal to 0.262 mg/kg bw per day
		Offspring toxicity	5 ppm, equal to 0.262 mg/kg bw per day[b]	—
	Developmental toxicity[c]	Maternal toxicity	2 mg/kg bw per day	6 mg/kg bw per day
		Embryo/fetotoxicity	2 mg/kg bw per day	6 mg/kg bw per day
	Acute neurotoxicity[c]	Toxicity	0.02 mg/kg bw	25 mg/kg bw
Rabbit	Developmental toxicity[c]	Maternal toxicity	0.1 mg/kg bw per day	0.3 mg/kg bw per day
		Embryo/fetotoxicity	0.9 mg/kg bw per day[b]	—
Dog	Combined from 13-week and 1-year studies[d]	Toxicity	0.09 mg/kg bw per day[b]	—

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

Estimate of acceptable daily intake for humans

0–0.0005 mg/kg bw

Estimate of acute reference dose

0.001 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 cadusafos

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 90–100%
Distribution	Extensive, highest levels in liver, fat, kidney and the lungs
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid, nearly complete within 48 h, mainly via urine
Metabolism in animals	Extensive, primarily via oxidation and cleavage
Toxicologically significant compounds (animals, plants and the environment)	Cadusafos
Acute toxicity	
Rat, LD_{50}, oral	30 mg/kg bw
Rabbit, LD_{50}, dermal	12 mg/kg bw
Rat, LC_{50}, inhalation	0.04 mg/l air
Rabbit, dermal irritation	Not an irritant
Rabbit, ocular irritation	Not an irritant
Guinea-pig, dermal sensitization	Not a sensitizer (Buehler)
Short-term studies of toxicity	
Target/critical effect	Erythrocyte cholinesterase inhibition (rat)
Lowest relevant oral NOAEL	0.067 mg/kg bw per day (rat)
Genotoxicity	
	Not genotoxic
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Erythrocyte cholinesterase inhibition and decreased locomotor activity (rat)
Lowest relevant NOAEL	1 ppm, equal to 0.045 mg/kg bw per day (rat)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects
Lowest relevant reproductive NOAEL	5 ppm, equal to 0.262 mg/kg bw per day, highest dose tested (rat)
Developmental target/critical effect	Skeletal findings at overtly maternally toxic doses (rat)
Lowest relevant developmental NOAEL	2 mg/kg bw per day (rat)
Neurotoxicity/delayed neurotoxicity	
	Organothiophosphorus compound, neurotoxic. No evidence of delayed neuropathy

Summary

	Value	Study	Safety factor
ADI	0–0.0005 mg/kg bw	Long-term study; rat	100
ARfD	0.001 mg/kg bw	Study of developmental toxicity; rabbit	100

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CHLOROTHALONIL

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Explanation

Chlorothalonil is the International Organization for Standardization (ISO)–approved common name for tetrachloroisophthalonitrile. Chlorothalonil (Chemical Abstracts Service [CAS] No. 1897-45-6) is a non-systemic foliar fungicide used to control a wide range of fungal diseases in a variety of crops.

Chlorothalonil was previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1974, 1977, 1978, 1979, 1981, 1983, 1985, 1987, 1990 and 1992. In 1990, an acceptable daily intake (ADI) of 0–0.03 mg/kg body weight (bw) was established based on the no-observed-adverse-effect level (NOAEL) of 3 mg/kg bw per day, identified in a 2-year study in dogs that was evaluated in 1974.

Chlorothalonil was re-evaluated by the present Meeting as part of the periodic review programme of the Codex Committee on Pesticide Residues. The present Meeting evaluated newly submitted studies, including mechanistic studies in rats into the effects of chlorothalonil on the kidneys and studies on SDS-3701 (4-hydroxy-2,5,6-trichloroisophthalonitrile), a metabolite that is found in plants, soil and ruminants. Both the new data and the relevant data from previous studies were considered by the present Meeting.

All critical studies complied with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

Mice

Male CD-1 mice (16 per dose) were treated by gavage with [^{14}C]chlorothalonil (radiochemical purity 98.2%) and chlorothalonil (purity 99.7%) at single doses of 0, 1.5, 15 or 105 mg/kg bw. The vehicle was 0.5% aqueous methyl cellulose. Urine and faeces were collected over 9, 24, 96 or 168 h periods. Four animals per dose were killed at 9, 24, 96 and 168 h, at which time blood and tissues were sampled. Statements of adherence to quality assurance (QA) and GLP were included.

The major route of elimination was via the faeces. Faecal elimination (97%, 85% and 75% at the low, middle and high doses, respectively) was almost complete by 24 h in the low and middle dose groups and by 96 h in the high dose group. Delayed elimination at the high dose was attributed to the presence of large amounts of radioactivity remaining in the stomach at 9 and 24 h. In the low and middle dose groups, 87% and 75% of the administered dose, respectively, were excreted within 9 h. At all doses, 5–10% of the administered radiolabel was excreted in urine. At termination, less than 3% of radiolabel was found in blood and tissues. Highest levels were found in the stomach. For the non-gastrointestinal tract tissues, the highest levels were found in the kidneys (Ribovich et al., 1983).

Rats

Bile duct–cannulated Sprague-Dawley rats (eight males and four females) received a single gavage administration of [^{14}C]chlorothalonil (radiochemical purity >95.5%) and chlorothalonil (purity 99.7%) at 5 mg/kg bw. The vehicle was 0.75% aqueous methyl cellulose. Half of the animals of each sex had biliary salt (taurocholate) infused. Bile was collected continuously. Blood was collected at 6, 24 and 48 h. Bile, urine and faeces were collected until 48 h post-dose. The gastrointestinal tract was separated from each carcass at termination. The sum of the radioactivity found in bile, urine and carcass was used to estimate the extent of absorption. Statements of adherence to QA and GLP were included.

About 31% of the dose was absorbed, independent of sex or biliary salt infusion. After 48 h, 21.1% and 7.8% (males) and 16.7% and 12.0% (females) of the administered dose were excreted in bile and urine, respectively. At 48 h, faecal excretion was 50% in males and 61% in females. Peak concentrations of radiolabel in blood and bile were observed at 6 h and 2 h after dosing, respectively. Compared with males, biliary excretion was 20% lower and urinary excretion was 50% higher in females (Marciniszyn et al., 1985a).

A single dose of [^{14}C]chlorothalonil (radiochemical purity >97.7%) and chlorothalonil (purity 99.7%) was administered to bile duct–cannulated male rats (six per dose) at 0, 1.5, 5, 50 or 200 mg/kg bw. The vehicle was 0.75% methyl cellulose. Bile samples were collected continuously for 48 h. Blood was collected from the tail vein at various time points after dosing, depending on the dose. Urine and faecal samples were collected until 48 h after dosing. At termination at 48 h, kidneys, stomach and small and large intestines were removed. Levels of radioactivity were determined in bile, blood, urine, faeces, gastrointestinal tract and carcass. Statements of adherence to QA and GLP were included.

At doses of 1.5, 5 and 50 mg/kg bw, 7–8% of the administered dose was excreted in urine and 16–22% was excreted in bile after 48 h. Less than 2% of the radiolabel was retained in the carcass. Thus, at doses up to 50 mg/kg bw, about 32% of the oral dose was absorbed. Urinary excretion and biliary excretion were 5% and 8%, respectively, at 200 mg/kg bw, suggesting that the maximum rate of absorption of chlorothalonil had been reached. Peak blood levels for the 1.5, 5, 50 and 200 mg/kg bw groups were reached at 2–6 h, 4 h, 6–10 h and 24 h, respectively (Marciniszyn et al., 1986).

Male Sprague-Dawley rats (10 per dose) were administered [^{14}C]chlorothalonil (radiochemical purity 97.0%) at a single dose of 5, 50 or 200 mg/kg bw by gavage. The vehicle was 0.75% aqueous methyl cellulose. Blood samples were collected at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 18, 24 and 30 h after dosing. Statements of adherence to QA and GLP were included.

Radiolabel was detected in blood within 30 min of administration of a single dose of [^{14}C]-chlorothalonil to rats by oral gavage. Peak levels were observed 6.1, 8.9 and 15.9 h after dosing in the low, middle and high dose groups, respectively, and calculated half-lives in blood were 6, 7.3 and >10 h, respectively (Pollock et al., 1983).

Male Sprague-Dawley rats (20 per dose) were administered [^{14}C]chlorothalonil (radiochemical purity 95.5–98.6%) at daily doses of 1.5, 5, 50 or 160 mg/kg bw by gavage on 5 consecutive days. The vehicle was 0.75% aqueous methyl cellulose. Four rats from each dose group were killed 2, 9, 24, 96 or 168 h after the last dose, at which time points blood and tissue samples were collected. Urine and faeces were collected at 24 h intervals and at necropsy. An additional eight rats per group were used to collect blood samples during the treatment period, 6 and 24 h after doses 1, 3 and 5. Statements of adherence to QA and GLP were included.

The times to peak concentrations in blood and kidneys increased with increasing dose. The data show that at 160 mg/kg bw per day, blood concentrations did not increase with repeated dosing, suggesting that saturation of blood occurred between 50 and 160 mg/kg bw per day. The percentage of urinary excretion decreased with increasing single and/or multiple doses (Savides et al., 1985).

In order to investigate whether biliary metabolites of chlorothalonil undergo enterohepatic recirculation, bile was collected for 6 h from male donor rats (n = 8) dosed orally with 5 mg/kg bw of [^{14}C]chlorothalonil (radiochemical purity 98%; purity 98%). The bile was subsequently injected intraduodenally into recipient male rats, which also had their bile ducts cannulated. The bile of the recipient rats was collected for 24 h.

In the donor rats, 0.6–3.2% of the administered dose was excreted in bile during the first 6 h after dosing. In the rats dosed intraduodenally with this bile, 15–25% of the administered radioactivity was excreted in bile during the first 24 h. These data suggest that enterohepatic recirculation plays a role in the metabolism and disposition of chlorothalonil in male Sprague-Dawley rats (Marciniszyn et al., 1983).

In order to investigate whether active transport processes are involved in renal excretion of radiolabel following administration of [^{14}C]chlorothalonil, 14 male rats were intraperitoneally pretreated with vehicle (corn oil) or probenecid (143 or 244 mg/kg bw), a potent inhibitor of various cellular transporters (including the organic anion transporter expressed on renal tubule cells), and subsequently orally dosed with [^{14}C]chlorothalonil (radiochemical purity 99%; purity 99.7%) at 50 or 5 mg/kg bw. Statements of adherence to QA and GLP were included.

Urinary excretion of radiolabel was decreased by 50% in probenecid-treated rats, compared with rats that received chlorothalonil alone (Savides, Marciniszyn & Killeen, 1985).

Male Sprague-Dawley rats (four per group) received a single oral administration by gavage of [^{14}C]chlorothalonil (radiochemical purity 99%) at 50 mg/kg bw. Control animals received 0.75% methyl cellulose. In order to investigate whether orally administered chlorothalonil becomes irreversibly bound to kidney macromolecules, deoxyribonucleic acid (DNA) and proteins were isolated from kidneys of these rats, which were killed 6 h after dosing. Animals treated intraperitoneally with [^{14}C]-dimethylnitrosamine (DMNA) were used as a positive control. Statements of adherence to QA and GLP were included.

Significant binding of radioactivity to proteins was observed, but covalent binding to DNA was not detected. DMNA treatment resulted in increased radiolabel binding to both protein and DNA (Savides, Marciniszyn & Killeen, 1987).

Male Sprague-Dawley rats (12 per group) received a single oral administration by gavage of [^{14}C]chlorothalonil (radiochemical purity 99%) at 50 mg/kg bw. The vehicle was 0.75% methyl cellulose. Six hours after administration, the animals were killed, and the kidneys were homogenized and fractionated. The radiolabel content of subcellular fractions was determined. Statements of adherence to QA and GLP were included.

The subcellular fractionation study demonstrated that 81% of the radiolabel was bound to the cytosolic fraction and that 54% of the remaining radiolabel was bound to the mitochondrial fraction (Savides et al., 1987).

Dogs

Four male bile duct–cannulated Beagle dogs were administered [^{14}C]chlorothalonil (radiochemical purity 99.5%) and chlorothalonil (purity 99.6%) at 50 mg/kg bw by gavage. The vehicle was

0.75% aqueous methyl cellulose. Urine, faeces, bile and cage washes were collected until 48 h after dosing, when the animals were killed. Liver, kidneys and blood were collected at termination. All samples were analysed for radiolabel by liquid scintillation counting or combustion liquid scintillation counting. Major biliary and urinary metabolites were analysed by extraction and chromatography. Statements of adherence to QA and GLP were included.

Two of the four animals vomited, causing loss of 7.7–9.0% of the administered dose. The data were corrected for this loss. Approximately 81% and 1.4% of the administered radiolabel were recovered from faeces and urine, respectively. In bile, 5.1% was excreted, with a peak excretion 10–14 h after dosing. Less than 0.1% of the administered dose was present in liver and kidneys. After 48 h, 0.06–0.4%, 0.08–1.35% and 0.01–0.20% of the administered dose were found in blood, muscle and fat, respectively. Bile and urine contained a large number of polar compounds (not identified), probably derived from reactions of chlorothalonil with glutathione (Savides et al., 1995).

(b) Dermal route

In vitro

The dermal absorption of chlorothalonil was investigated in vitro using skin from Wistar rats and human skin. [^{14}C]Chlorothalonil (radiochemical purity >95%) and chlorothalonil (purity 99.5%) were applied at concentrations of 7, 0.037 and 0.0046 mg/cm^2. All applications remained unoccluded for the duration of the 24 h exposure period.

The radioactivity in skin, receptor fluid and equipment was analysed using liquid scintillation counting. Statements of adherence to QA and GLP were included.

After 24 h, the majority of the radiolabel could be washed off both the rat and human skin. After 24 h, 0.04%, 6.8% and 16.5% of the applied dose were absorbed following application of the high, intermediate and low concentrations to rat skin, respectively. For human skin, the corresponding percentages were 0.02%, 0.05% and 0.28%, respectively (Davies, 2000a,b).

In vivo

A suspension concentrate formulation containing [^{14}C]chlorothalonil (radiochemical purity >98%) was applied to 10 cm^2 of the shaven dorsal skin of male Alpk:AP$_f$SD (Wistar derived) rats at doses of 34 (undiluted formulation) or 0.36 and 0.042 mg (aqueous dilutions) of chlorothalonil per rat. Accordingly, skin concentrations of chlorothalonil were 3.4, 0.036 and 0.0042 mg/cm^2. The application sites were covered with gauze. Groups of four rats were exposed for 10 and 24 h or for 10 h followed by an interim skin wash, with these rats kept alive until 24 h after dosing. At the end of each period, the rats were anaesthetized and the skin was washed, before exsanguination. The protective covers were removed, and the application site skin was excised and tape-stripped to remove the stratum corneum. Radioactivity in skin samples, tape strips, skin wash sponges, blood, plasma, gastrointestinal tract (including contents), carcasses, urine and faeces was measured by liquid scintillation counting. Statements of adherence to QA and GLP were included.

During exposure of rats for up to 24 h, the in vivo dermal absorption of [^{14}C]chlorothalonil at a concentration of 3.4 mg/cm^2 (undiluted suspension concentrate formulation) was 0.7%. Absorption at concentrations of 0.036 and 0.0042 mg/cm^2 was approximately 2% and 10%, respectively. Of the absorbed dose, the majority appeared to be excreted via the bile in the faeces (Jones, 2000).

1.2 Biotransformation

Rats

A single oral dose of [^{14}C]chlorothalonil (radiochemical purity >99%) at 250 mg/kg bw was administered by gavage to 12 male Fischer 344 rats. Samples of urine and faeces were obtained at 6,

12, 24, 48 and 72 h. Metabolites of chlorothalonil in urine were identified by high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC-MS). Statements of adherence to QA and GLP were included.

Excretion of radiolabel in urine and faeces occurred primarily between 6 and 24 h. Within 48 h, 93% and 2.5% of the administered dose were excreted in faeces and urine, respectively. In the urine, eight major metabolites were identified. It is proposed that chlorothalonil is metabolized via initial glutathione conjugation and subsequent enzymatic processing of the diglutathione and triglutathione substituents via the mercapturic acid and cysteine conjugate β-lyase pathway, yielding *N*-acetyl cysteine, cysteinyl-glycine and *S*-methyl derivatives. The proposed metabolic pathway for urinary metabolites is presented in Figure 1 (Brady & Kenna, 1999).

The LC-MS analysis performed by Brady & Kenna (1999) did not confirm the presence of dithiol or trithiol metabolites, which were found by gas chromatography–mass spectrometry (GC-MS) analysis in studies by Savides et al. (1986) and Marciniszyn et al. (1985b). Therefore, the urine samples from the study of Brady & Kenna (1999) were further analysed by Cuff, Kenna & Withe (2001). The urine samples were extracted with ethyl acetate and analysed by GC-MS, HPLC and LC-MS. The study demonstrated that metabolites detected by LC-MS are not chemically stable when analysed by GC-MS, even after derivatization with diazomethane, and break down to form the dithiols and trithiols. These artefacts are readily methylated to give the corresponding *S*-methyl derivatives (Cuff, Kenna & Withe, 2001).

Based on the findings of the study of Cuff, Kenna & Withe (2001), the Meeting concluded that the metabolism studies using GC-MS only cannot be deemed sound, and they were therefore not evaluated.

Figure 1. Proposed pathway of formation of urinary metabolites in the rat (Brady & Kenna, 1999)

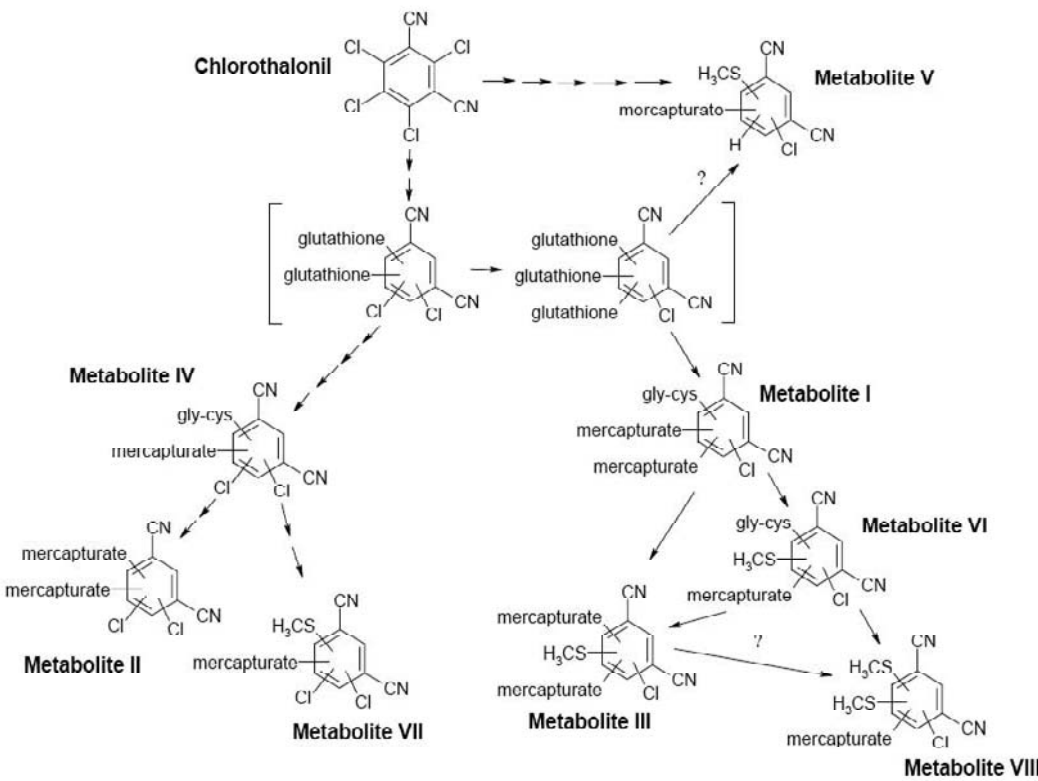

Table 1. Results of LD_{50}/LC_{50} studies with chlorothalonil

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw) or LC_{50} (g/m^3)	Reference
Rat	Sprague-Dawley	Male/ female	Oral	Aqueous carboxymethyl cellulose	98.6	>5000	Moore (2000)[a,b]
Rat	Alpk:AP$_f$SD	Male/ female	Dermal	Water	98.6	>5000	Johnson (2000a)[a,c]
Rat	Sprague-Dawley	Male/ female	Inhalation	Air	98.2	0.10	Shults, Brock & Laveglia (1993)[a,d]

[a] Statements of adherence to GLP and QA were included.
[b] Performed according to Office of Prevention, Pesticides and Toxic Substances (OPPTS) 870.100, which resembles Organisation for Economic Co-operation and Development (OECD) test guideline 401.
[c] Performed according to OECD test guideline 402.
[d] Performed according to United States Environmental Protection Agency (USEPA) Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guideline 81-3, with whole-body exposure. The mass median aerodynamic diameter (MMAD) was 2.5–3.6 µm. Clinical signs during exposure were eyes partially closed, gasping, exaggerated respiratory movements, restless behaviour, wet around the eyes and mouth, and clear discharge from snout. After exposure, exaggerated respiratory movements, wet fur, gasping and discharge from the eyes were seen.

Dogs

Three male Beagle dogs were administered [^{14}C]chlorothalonil (radiochemical purity 99%) and chlorothalonil (purity 98.9%) at 50 mg/kg bw by gavage. The vehicle was 0.75% aqueous methyl cellulose. In two of the dogs, urine was collected via a urinary catheter for 24 h. Subsequently, in all three animals, samples of urine and faeces were taken at 24 h intervals up to 72 h. Samples of urine and faeces were analysed for radioactivity. Urine samples that contained sufficient amounts of radioactivity for further analyses were acidified and extracted with organic solvent. The organic extracts were concentrated, methylated, separated by HPLC and analysed by GC-MS. Statements of adherence to QA and GLP were included.

Recovery of radiolabel in urine ranged from 0.2% to 2.4% of the administered doses. Recovery from faeces was approximately 94%. No monomethylthio or dimethylthio analogues of the thiols were detected. One sample of urine contained the trimethylthio analogue (0.000 12% of the administered dose) (Magee et al., 1991).

2. Toxicological studies

2.1 Acute toxicity

The results of acute lethal dose (LD_{50}) and acute lethal concentration (LC_{50}) studies with chlorothalonil are summarized in Table 1.

(a) Oral administration

In a preliminary acute toxicity study, chlorothalonil (purity 98.9%; batch No. FL041352) was administered by gavage in 1.0% aqueous carboxymethyl cellulose suspension at 0, 20, 180 or 1000 mg/kg bw as a single dose to Fischer 344 rats (10 of each sex per dose). In this study, the toxic effects of chlorothalonil and the effects on cell proliferation in the kidney were investigated. For toxicity testing at 24 and 96 h, two animals of each sex per group were killed. To study the effect of chlorothalonil on cell proliferation, three animals of each sex per group were sacrificed at about 24 and 96 h. Seven days before scheduled termination, cell proliferation animals were implanted with

minipumps, which delivered bromodeoxyuridine (BrdU) for the duration of the study. At termination, the number of BrdU-labelled cells in the kidney was counted by an automated method. For toxicity-phase animals, clinical observations, body weights and food consumption were recorded daily, and urine samples for urinalysis (parameters measured were not reported) were collected overnight on the day prior to termination. All rats were examined macroscopically, and kidney weights were recorded. The kidneys were examined by light microscopy.

There were no compound-related changes in body weights, food consumption or urine clinical chemistry parameters. Clinical observations were limited to piloerection at 1000 mg/kg bw on the day of dosing. There were no treatment-related effects on kidney weights or macroscopic pathological findings. One mid-dose and two high-dose females killed at 96 h showed minimal proximal tubular vacuolation. No differences in the percentages of BrdU-labelled cells between treated and control animals were found (Lees, 2005a).

Note: In the study report, only a short description of the findings is presented; no actual data were reported.

In a follow-up study, chlorothalonil (purity 98.9%; batch No. FL041352) was administered by gavage in 1.0% aqueous carboxymethyl cellulose suspension at single doses of 0, 20, 60 or 250 mg/kg bw to Fischer 344 rats. Groups of five rats of each sex per dose per time point for either toxicity testing or cell proliferation testing were killed at approximately 24 h (day 2) or 96 h (day 5) after dosing. Cell proliferation was tested as described above (Lees, 2005a). For toxicity testing of animals, a detailed clinical examination was performed prior to dosing and at 0.5, 2 and 4 h after dosing and subsequently at least once per day. In these groups, haematology, clinical chemistry and urinalysis were performed at termination. For all groups, body weights and food consumption were recorded daily. All rats were examined macroscopically, and kidney weights were recorded. The kidneys and all tissues with lesions were examined by light microscopy. Kidneys and duodenum of the cell proliferation groups were examined for number of BrdU-labelled and unlabelled cells. Statements of adherence to QA and GLP were included.

There were no treatment-related changes in body weight, food consumption, urinalysis or haematology. In all treatment groups, clinical chemistry revealed lower levels of alkaline phosphatase (AP) (males), alanine aminotransferase (ALAT) (both sexes) and aspartate aminotransferase (ASAT) and creatine kinase activities (females). However, reductions in levels of these enzymes are generally not considered to be adverse. In the absence of treatment-related effects on kidney weights, macroscopy or histopathology, other isolated changes in clinical chemistry are considered not to be of toxicological significance. No differences in the percentages of BrdU-labelled cells between treated and control animals were found.

The NOAEL in this study was 250 mg/kg bw, the highest dose tested (Lees, 2005b).

(b) Dermal irritation

In a dermal irritation study, performed according to Organisation for Economic Co-operation and Development (OECD) test guideline 404, three male New Zealand White rabbits were dermally exposed for 4 h to 0.5 g of chlorothalonil (purity 98.6%; lot No. P5; solid powder) moistened with deionized water. Dermal irritation was scored according to the Draize system at 1, 24, 48 and 72 h and subsequently at intervals up to 10 days after patch removal. Statements of adherence to QA and GLP were included.

Very slight erythema was seen in two animals for 1 h or 5 days. Very slight to moderate/severe erythema was observed in one animal for 7 days. Very slight oedema was observed in one animal for 2 days, and very slight to slight oedema was observed in another animal for 3 days. Additionally, desquamation, thickening, scabbing and wrinkling of the skin were observed. All signs had resolved by day 10. It is concluded that chlorothalonil is a mild skin irritant (Johnson, 2000b).

(c) Ocular irritation

In an eye irritation study, 0.1 g of chlorothalonil (purity 99.6%; batch No. 9059-90-3; solid powder) was instilled into the conjunctival sac of one eye of young adult male and female New Zealand White rabbits (three of each sex). The untreated eye served as a control. Eye irritation was scored according to the Draize system at 24, 48 and 72 h and on days 7 and 14 post-instillation.

Corneal opacities were observed in all treated eyes at all observation intervals, with the exception of five animals at 24 h and four animals at 48 h, when corneas could not be observed as a result of severe chemosis. Pannus was observed in two eyes at day 7. Dendritic vascularization was observed in two eyes at day 7. Vascularization of the corneal surface was observed in five eyes at day 14. Peeling of the corneal epithelium and iritis were observed in all animals. Conjunctival redness, chemosis, discharge and blanching were noted in all animals at all intervals. Petite conjunctival haemorrhages were observed in all animals through day 7.

It is concluded that chlorothalonil is severely irritating to the eye (Major, Killeen & Dean, 1982).

(d) Dermal sensitization

In a study to evaluate the sensitizing potential of chlorothalonil (purity 98.1%), three experiments (two maximization tests and one open epicutaneous test) were conducted using Dunkin Hartley guinea-pigs. However, it was concluded that the sensitization potential of chlorothalonil could not be accurately evaluated using the results of these experiments (Wilson et al., 1988).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 3-month feeding study, chlorothalonil (purity 98.4%) was administered to CD-1 mice (15 of each sex per concentration) in the diet at 0, 7.5, 15, 50, 275 or 750 ppm, equal to 0, 1.2, 2.5, 8.5, 47.7 and 123.6 mg/kg bw per day for males and 0, 1.4, 3.0, 9.8, 51.4 and 141.2 mg/kg bw per day for females. Animals were observed twice daily for mortality and clinical signs. A detailed clinical examination and body weight measurement were performed weekly. Food consumption was measured twice per week. Blood was collected at week 6 (five mice of each sex per dose) and at termination (all remaining animals) for clinical chemistry tests. After the collection of blood, the animals were killed and necropsied. Weights of brain and kidneys were recorded. The stomach and kidney of all animals were examined histologically. Statements of adherence to QA and GLP were included.

Treatment had no effect on mortality or clinical signs. Mild increases in body weight in males of some of the treatment groups were attributed to a temporary body weight loss in the control group and were considered not toxicologically relevant. A mild but statistically significant increase (45%) in AP was observed in high-dose females, whereas ALAT levels were decreased. No treatment-related macroscopic changes were observed. At the interim kill at week 6, an increase in relative kidney weight (15%, not statistically significant) was observed in the high-dose males. At the terminal kill, relative kidney weight was statistically significantly increased (13%) in the females of the 750 ppm group. In both sexes, hyperplasia and hyperkeratosis of the squamous epithelial cells of the forestomach were observed in the 275 and 750 ppm groups at the interim kill and in the 50, 275 and 750 ppm groups at the terminal kill. In the highest dose group, an increased incidence of epithelial hyperplasia in the kidneys was observed in both sexes.

The NOAEL for systemic effects was 275 ppm, equal to 47.7 mg/kg bw per day, based on an increased incidence of epithelial hyperplasia in the kidneys and increased kidney weight at the high

dose. The NOAEL for local effects is 15 ppm, equal to 2.5 mg/kg bw per day, based on hyperplasia and hyperkeratosis of the squamous epithelial cells of the forestomach (Shults & Killeen, 1983; Shults, Wilson & Killeen, 1985).

Rats

In a 4-week oral study in Fischer 344 rats, which was performed as a range-finding study for a carcinogenity study, chlorothalonil (purity 98%) was administered in food at 0, 960, 2060, 4600, 9200 or 17 100 ppm in males and at 0, 870, 1820, 4460, 8690 or 14 760 ppm in females. These dietary levels corresponded to about 0, 80, 175, 375, 750 and 1500 mg/kg bw per day in both sexes. Groups consisted of 10 animals of each sex per dose. Clinical observation data were collected at least once daily. Body weight, food consumption and detailed clinical observations were recorded weekly. At termination, haematology and ALAT measurements were conducted, and the animals were examined macroscopically. Liver and kidney weights were recorded. Statements of adherence to QA and GLP were included.

One male at 1500 mg/kg bw per day died during the first week of treatment. Although no details about the cause of death were reported, the study authors considered the death to be treatment related. At doses of 375 mg/kg bw per day and higher, increases in clinical signs (generally poor physical condition, soft stool, mucus in stool, red nasal discharge, dark crusty material around the nose, decreased faecal output, swelling and/or irritation around the anus) were observed. The severity of the clinical signs increased with the dose. At doses of 175 mg/kg bw per day (males) or 375 mg/kg bw per day (females) and higher, dose-dependent decreases in body weight and food consumption were observed. Reductions in erythrocyte count, haematocrit and haemoglobin were observed at the three highest doses. At the two highest doses, leukocyte counts were reduced. ALAT levels were dose-dependently reduced in all treatment groups. These decreases are not considered toxicologically relevant. In both sexes in all treatment groups, increases in relative weights of liver (9–31%) and kidney (9–45%) were found.

Based on the increases in relative liver and kidney weights, the lowest-observed-adverse-effect level (LOAEL) was 80 mg/kg bw per day (Wilson, Killeen & Ignatoski, 1982).

Charles River CD rats (20 of each sex per dose) received chlorothalonil (purity 98%) in the diet for 90 days. The concentrations of test compound in the diet were changed as necessary to obtain doses of 0, 40, 80, 175, 375, 750 or 1500 mg/kg bw per day. Clinical observation data were collected twice daily. A detailed clinical examination was carried out once per week. Body weight and food consumption were recorded weekly. Haematology, clinical chemistry and urinalysis were performed on 10 animals of each sex per dose after 1 month and at termination. At termination, animals were necropsied. Weights of brain, heart, kidneys, liver and testes/ovaries were recorded, and a full range of tissues was microscopically examined. Statements of adherence to QA and GLP were included.

At 750 and 1500 mg/kg bw per day, mucus in stools, red nasal discharge or dark crusty material around the nose, decreased faecal output, swelling and/or irritation of the area around the anus and generally poor condition were observed. Statistically significant and dose-dependent decreases in body weight gain (14–35%) were observed at doses of 375 mg/kg bw per day and higher. At doses of 175 mg/kg bw per day and higher, food consumption was statistically significantly and dose-dependently decreased during week 1 of treatment, whereas food consumption in these treatment groups generally was higher from the second week onward. No treatment-related changes in haematological parameters were noted. Significant reductions in blood glucose at 375 mg/kg bw per day and higher, blood urea nitrogen (BUN) at 80 mg/kg bw per day and higher and thyroxine at 175 mg/kg bw per day and higher were observed. ALAT levels were reduced in all treatment groups. Urine volume was decreased and specific gravity was increased in males dosed at 375 mg/kg bw per day and higher. Dose-related increases in absolute and relative kidney weights were observed at all dose levels. Hyperplasia was

noted in the proximal tubules of male rats at all dose levels and in females at 175 mg/kg bw per day and higher. A higher incidence of karyomegaly occurred in kidneys of males of all treatment groups.

The LOAEL was 40 mg/kg bw per day, the lowest dose tested, based on increased kidney weights in both sexes and histological changes in the kidneys in males (Wilson & Killeen, 1981; Wilson, Killeen & Ignatoski, 1985).

In a 13-week feeding study with a 13-week recovery period, chlorothalonil (purity >97.7%) was administered to Sprague-Dawley rats (25 of each sex per dose). Some of the animals received control food from week 13 to week 26. The concentrations of test compound in the diet were changed as necessary to obtain doses of 0, 1.5, 3.0, 10 or 40 mg/kg bw per day. Actual compound intake was 0, 1.5, 3.0, 10.3 and 40.7 mg/kg bw per day in males and 0, 1.5, 3.1, 10.2 and 40.7 mg/kg bw per day in females. Clinical observation data were collected at least once daily for the first 4 weeks of treatment and once a week thereafter. Body weight, food consumption and water consumption were recorded weekly. For the purpose of haematology, clinical chemistry and urinalysis, blood and urine were collected from 10 animals of each sex per dose at weeks 6 and 13 and at weeks 19 and 26, respectively, from all surviving animals. Faecal samples were collected from five animals of each sex per dose at weeks 6, 13, 19 and 26 in order to study the effect of treatment on the faecal microflora. Animals were killed for necropsy at week 6 (five of each sex per dose), week 13 (10 of each sex per dose) and week 26 (all surviving animals). At these designated time points, the animals were macroscopically examined, weights of brain, heart, kidneys, liver and testes/ovaries were recorded and a full range of tissues was examined by light microscopy. Kidney tissue was also examined by electron microscopy. Statements of adherence to QA and GLP were included.

During the study, six animals died or were killed. None of the deaths was related to treatment. No treatment-related clinical signs were observed. Treatment did not affect body weight gain or food and water consumption. Decreases in AP and ALAT were observed at 13 weeks in females of the high dose group and in males of all treated groups. However, these decreases are considered not toxicologically relevant. Urinalysis, urine concentration tests and the microbiological assay did not show an effect of treatment. No macroscopic changes were observed. At 10 and 40 mg/kg bw per day, histopathology revealed increased incidences of epithelial hyperplasia and hyperkeratosis in the non-glandular epithelium of the stomach. Kidney weights were slightly but statistically significantly increased (13–15%) in males of the 10 and 40 mg/kg bw per day groups after 6 weeks of treatment and in both sexes of the 3, 10 and 40 mg/kg bw per day groups (12–20%) after 13 weeks of treatment. After the 13-week recovery period, no differences in kidney weight were observed. Examination by light microscopy revealed hyperplasia of the epithelium of proximal convoluted tubules in males at 40 mg/kg bw per day, which was irreversible. Electron microscopy and light microscopy revealed increased incidences and severity of irregular intracytoplasmic inclusion bodies in proximal tubules in males at all dose levels, which were only partially reversible during a 13-week recovery period. As these inclusions were not found in control or chlorothalonil-treated females, the toxicological significance of this finding is not clear.

In this study, the NOAEL is considered to be 10 mg/kg bw per day, based on hyperplasia and increased kidney weights at 40 mg/kg bw per day (Colley et al., 1983; Wilson & Killeen, 1983a, 1985a; Wilson, Killeen & Haley, 1984).

Dogs

In a 90-day feeding study performed in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guideline 82-1 of the USA, which resembles OECD test guideline 408, chlorothalonil (purity 97.9–98.2%) was administered by capsule to Beagle dogs (four of each sex per dose) at 0, 15, 150 or 750 (500) mg/kg bw per day. As a result of one mortality and severe emesis, the high dose was reduced to 500 mg/kg bw per day on day 5 of treatment. All animals were observed

at least twice daily for mortality and clinical signs. Detailed physical examinations were conducted weekly. Body weight was measured weekly, and food consumption was measured 4 times per week. Ophthalmoscopy was performed prior to the start of the study and prior to termination. Blood and urine samples were collected pretest, at 1.5 months and at 3 months for haematology, clinical chemistry and urinalysis. At termination, the dogs were killed and subjected to gross examinations. Selected organs were weighed. An extensive range of organs was examined histologically. Statements of adherence to QA and GLP were included.

At 750 mg/kg bw per day, one male died due to aspiration of vomit. The surviving high-dose dogs displayed severe vomiting, which returned to control levels after lowering the dose to 500 mg/kg bw per day. Statistically significant decreases in body weight gain over the dosing period were observed in males of the middle (−56%) and high dose (−60%) groups. Clinical chemistry showed statistically significantly increased levels of cholesterol at 1.5 and 3 months in both sexes at the high dose (up to 68%) and in females of the middle dose group (up to 52%). Statistically non-significant and non-dose-dependent increases in AP were observed in both sexes at the high dose (up to 51%) and in females in the middle dose group (up to 72%). Statistically significant reductions in the levels of albumin (mid-dose males −9%; both sexes at the high dose up to −15%) and ALAT (both sexes of all treatment groups) were observed. The reduction in ALAT is not considered adverse. Relative liver weight was significantly increased (up to 31%) in both sexes in the high dose group. No substance-related effects on ophthalmoscopy, haematology, urinalysis, or gross and microscopic pathology were observed.

The NOAEL was 15 mg/kg bw per day, based on effects on body weight gain in males and changes in clinical chemical parameters (Fillmore & Laveglia, 1993).

In a 1-year study, performed in accordance with OECD test guideline 409, chlorothalonil (purity 97.9%; lot No. 1002) was administered by capsule to Beagle dogs (five of each sex per dose) at 0, 15, 150 or 500 mg/kg bw per day. All animals were observed at least twice daily for mortality and clinical signs. Detailed clinical examinations were conducted weekly. Body weight was measured weekly. Food consumption was checked at least 4 times per week. Ophthalmoscopy was performed prior to the start of the study and prior to termination. Blood and urine samples were collected pretest and at weeks 13, 26 and 52 for haematology, clinical chemistry and urinalysis. At termination, the dogs were killed and subjected to gross examinations. Brain, kidneys, liver and testes with epididymides were weighed. An extensive range of organs was examined histologically. Statements of adherence to QA and GLP were included.

No substance-related effects on survival, clinical signs or ophthalmology were observed. At termination, body weights of high-dose males and females were 15–16% lower than those of controls. In the high-dose animals, body weight gain over the treatment period was lower (48–52%) than in control animals, reaching statistical significance in the females. Food consumption was not affected by treatment. No effects of treatment on haematology were observed. Decreases in reticulocyte counts in the mid- and high-dose animals (1.0–1.9%) were attributed to relatively high control values (1.5–2.4%), which were outside the historical control range of 0.5–1.9%. Statistically significant reductions in ALAT (both sexes of all treatment groups) were considered not adverse. Levels of cholesterol were increased at 6 months (71%, statistically significant) and 12 months (41%, not statistically significant) in females of the high dose group. Total protein levels were decreased in high-dose males, and lower albumin levels were observed in both sexes at the high dose. Relative liver weights were statistically significantly increased in both sexes in the middle dose (22–26%) and high dose (22–50%) groups. These increased liver weights were not accompanied by histological changes in the liver. Relative kidney weights were increased in females at the middle dose (22–24%, not statistically significant) and high dose (27%, statistically significant), but not in males. A slight, dose-related increase in the incidence and severity of kidney pigmentation in both sexes was observed. This was not accompanied by histopathological changes in the kidney.

The NOAEL was 150 mg/kg bw per day, based on the reduced body weight gain, reduced serum albumin, increased relative liver weight, lower serum total protein in males and higher cholesterol in females at the high dose (Mizens & Laveglia, 1994).

(b) Dermal administration

Rats

In a 21-day dermal study, chlorothalonil (purity 98.1%; lot No. 313012) was applied under occlusion to the shaved, intact dorsal skin of Fischer 344 rats (10 males per dose). The vehicle was 0.2% aqueous methyl cellulose. The rats were exposed to the test substance under semiocclusion for 6 h/day, 5 days/week, for 3 weeks. Exposure doses were 0, 60, 100, 250 and 600 mg/kg bw per day.

All animals were observed daily for mortality and clinical signs. The application sites were scored daily for erythema, eschar and oedema according to the Draize method. Body weight and food consumption were recorded weekly. At termination, blood samples were collected for clinical chemistry, and the animals were subjected to gross examinations. Brain and kidneys were weighed. Forestomach, kidneys, treated and untreated skin and gross lesions were examined histologically. Statements of adherence to QA and GLP were included.

No mortality occurred. From day 10 of treatment, rough coat (250 and 600 mg/kg bw per day) and coloured material around the nose (high dose) were observed. In all treatment groups, erythema (first observed at day 8) and desquamation (first observed at day 10) occurred. The application sites had a yellow colour. Body weight gain was reduced in the high dose group. A slight but statistically significant reduction in food consumption (up to 8%) was observed in the middle and high dose groups during the first week of treatment. Levels of ALAT were decreased at 250 and 600 mg/kg bw per day. Relative kidney weights were dose-dependently increased (9–18%) in all dose groups. The study author attributes the increase to low kidney weights in the control group, with reference to kidney data from another contemporary study from the same laboratory. The present reviewers endorse this view. No histopathological effects in the kidneys or forestomachs were observed. Histological examination of the treated skin showed hyperkeratosis and squamous epithelial hyperplasia at all doses.

Based on the reduction in body weight gain at 600 mg/kg bw per day, the NOAEL for systemic effects is 250 mg/kg bw per day. The LOAEL for local skin effects is 60 mg/kg bw per day, the lowest dose tested (Mizens, 1996).

Rabbits

In a 21-day dermal study, chlorothalonil (purity 98.4%; batch No. 1002) was applied under occlusion to the shaved, intact dorsal skin of New Zealand White rabbits (six of each sex per dose). The vehicle was 0.125% aqueous methyl cellulose. The rabbits were exposed to the test substance under semiocclusion for 6 h/day for 21 days. Exposure doses were 0, 0.1, 2.5 and 50 mg/kg bw per day. All animals were observed daily for mortality and clinical signs. The application sites were scored daily for erythema, eschar and oedema, according to the Draize method. A detailed physical examination was carried out weekly. Body weight and food consumption were recorded weekly. At termination, blood and urine samples were collected for haematology, clinical chemistry and urinalysis, including metabolite analysis, and the animals were subjected to gross examinations. Brain, kidneys, liver, adrenal glands and gonads were weighed. An extensive number of tissues were histologically examined. Statements of adherence to QA and GLP were included.

No treatment-related mortality or clinical signs were found. Erythema/oedema was observed in females of the low dose group and in both sexes of the middle and high dose groups. Desquamation was observed in both sexes at the middle and high doses. Acanthosis and hyperkeratosis of the epidermis were observed in skin samples from the 2.5 and 50 mg/kg bw per day groups. Body weight gain, food consumption and haematology were not affected by treatment. Levels of ALAT were decreased at 2.5 and 50 mg/kg bw per day. Relative kidney weights were increased in males of the middle dose

group and in both sexes at the high dose. Histological examination of organs was confounded by the occurrence of a parasitic infection in the animals (Shults & Wilson, 1986).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a 2-year carcinogenicity feeding study, groups of CD-1 mice (60 of each sex per dose) were fed diets containing chlorothalonil (purity 97.7%) at 0, 750, 1500 or 3000 ppm, equal to 0, 119, 252 and 517 mg/kg bw per day in males and 0, 134, 279 and 585 mg/kg bw per day in females. Animals were checked twice daily for clinical signs, and a detailed clinical examination was performed weekly. Body weights and food consumption were recorded weekly for the first 14 weeks, every other week through to week 26 and every month thereafter. Haematology was performed in 10 mice of each sex per dose at 12 and 18 months and at termination. At termination, gross examination was performed on all animals. Selected organs were weighed. An extensive number of tissues were examined microscopically. Statements of adherence to QA and GLP were included.

No treatment-related effects on mortality, clinical signs, body weight gain and food consumption were observed. There were no statistically significant differences in haematology parameters, although a higher incidence and severity of altered red blood cell morphology were observed in the treatment groups. The toxicological significance of this finding is not clear. Statistically significant increases (up to 43%) in absolute and relative kidney weights were observed in all treatment groups. In all treatment groups, increased incidences of renal enlargement, discoloration, surface irregularities, pelvic dilatation, cysts, nodules and masses were found. An increased incidence of masses or nodules in the forestomachs of treated animals was also noted. Histological evaluation revealed glomerulonephritis, cortical tubular degeneration, cysts and hyperplastic lesions in the proximal convoluted tubules in both sexes of all treatment groups. These findings occurred more frequently in males than in females. In males, tubular adenomas and carcinomas were observed at all doses, whereas no kidney tumours were found in control males. There was an increase in the incidence of forestomach tumours, mainly squamous cell carcinomas, in the forestomach at all doses. In addition, hyperplasia and hyperkeratosis of the squamous mucosa in the forestomach were observed in all treatment groups. An increased incidence of hyperkeratosis was noted in the oesophagus of all treatment groups.

In this study, the LOAEL was 750 ppm, equal to 119 mg/kg bw per day, the lowest dose tested, on the basis of effects on kidney weights, macroscopic changes in kidney and forestomach, and microscopic changes in kidney, forestomach and oesophagus. Chlorothalonil induced kidney (males only) and forestomach tumours at all doses (Wilson & Killeen, 1983b, 1986a,b).

In a 2-year carcinogenicity feeding study, chlorothalonil (purity 98.0%; lot No. 177) was administered to CD-1 mice (60 of each sex per dose) at 0, 10/15 (increased to 15 ppm in week 18), 40, 175 or 750 ppm, equal to 0, 1.9, 5.1, 22.5 and 98 mg/kg bw per day. Animals were checked twice daily for clinical signs, and a detailed clinical examination was performed weekly. Body weights and food consumption were recorded weekly for the first 14 weeks and every other week thereafter. Haematology was performed in 10 mice of each sex per dose at 12 and 18 months and at termination. The 10 animals of each sex per dose selected for haematological examination at 12 months were subsequently necropsied. At termination, gross examination was performed on all animals. Brain and kidneys were weighed. The kidneys, stomach and related lymph nodes were examined microscopically. Statements of adherence to QA and GLP were included.

No treatment-related effects on mortality, clinical signs, body weight gain or food consumption were observed. At 12 months, but not at 18 or 24 months, statistically significant decreases (12%) in haemoglobin levels and haematocrit were observed in the high dose group. In the high dose group,

relative kidney weights were increased (up to 26%) at 12 and 24 months, associated with an increased incidence of granular appearance at 24 months. The incidences of tubular hyperplasia and karyomegaly were dose-dependently increased at 175 and 750 ppm. Increased incidences of hyperplasia and hyperkeratosis of the squamous epithelium of the forestomach were observed at 40 ppm and higher. A slightly higher incidence of forestomach tumours (total of papillomas and carcinomas) was observed at 750 ppm (Wilson & Killeen, 1987).

Rats

In a combined chronic toxicity and carcinogenicity feeding study, chlorothalonil (purity 98.1%) was administered in the diet to Fischer 344 rats (60 rats of each sex per dose) for approximately 116 weeks in males and 129 weeks in females. Concentrations of chlorothalonil in the diet were adjusted based on body weight and food consumption data in order to achieve the desired intake of 0, 40, 80 or 175 mg/kg bw per day. Actual mean intakes were 0, 40.5, 81.1 and 177.5 mg/kg bw per day in males and 0, 40.1, 80.3 and 175.6 mg/kg bw per day in females. The physical condition of the animals was monitored weekly. Body weight and food consumption were measured weekly for the first 14 weeks and once every 2 weeks thereafter. For haematological and clinical chemistry examinations, blood samples were collected from 10 animals of each sex per group after 12 (haematology only), 18 and 24 months and at termination. Urinalysis was performed at 10, 11, 18 and 24 months and at termination. Pooled urine samples were collected at 11, 14, 18 and 25 months to investigate the cause of dark-coloured urine observed in the high dose group. All rats were subjected to gross pathology, and selected organs were weighed. A wide range of tissues was evaluated microscopically. Statements of adherence to QA and GLP were included.

For the first 24 months, no treatment-related effect on mortality was observed. After 24 months, an increased mortality (not statistically significant) was observed in the high-dose males. From 8 to 21 months, urine of the high-dose animals was darker yellow than that of control animals. Statistically significant reductions in body weight gain were observed in all treatment groups. In low-dose males and females, these reductions were generally less than 10% throughout the study, whereas at the middle and high doses, they reached 10–15% and 25–29%, respectively.

Food consumption in high-dose animals was generally higher than in controls. Treatment-related changes in haematological parameters were probably related to a relatively high incidence of leukaemia in the control animals. A number of changes in clinical chemistry parameters were observed. Statistically significant reductions in ALAT and AP (both sexes of all treatment groups) were considered not adverse. Statistically significant increases in serum BUN levels in the males of the middle and high dose groups and in creatinine levels in high-dose males were considered to be the consequence of impaired kidney function at these doses. Statistically significant decreases in serum albumin were observed in males of the middle and high dose groups and in females of the high dose group. Statistically significant decreases in blood glucose levels (up to 12%) were observed in males of the middle and high dose groups. Cholesterol levels were statistically significantly increased in females of all treatment groups by 19%, 22% and 37%, respectively. Urinalysis showed an increased volume and decreased specific gravity in all treatment groups. In the high dose group, urine pH (females only) and ketone levels were higher.

Absolute and relative kidney weights were increased in all treatment groups (at the low dose only in males). In view of the observed effects on urinalysis and the macroscopic and histopathological changes in the kidneys (see below), the increased kidney weights were considered to be a direct consequence of chlorothalonil treatment. Relative heart, brain, liver, ovaries and testes weights generally were higher in the treatment groups. However, as these effects on organ weight were not accompanied by histological changes, they are considered to be secondary to the reduction in body weight gain.

Macroscopic examination revealed a dose-related increase in incidence and severity of the granularity of the kidney surface and in erosions and ulcerations of the non-glandular stomach in all treatment groups. In males of all treatment groups and in females of the high dose group, increased incidences

of kidney masses/nodules and parathyroid enlargement were observed. Histopathological examination revealed treatment-related effects on the kidneys and on the gastrointestinal tract (oesophagus, stomach, duodenum). In all treatment groups, dose-dependent increases in incidence and/or severity of chronic glomerulonephritis, tubular cysts and focal tubular epithelial hyperplasia were observed. In males of all dose groups, papillary/pelvic hyperplasia was dose-dependently increased. In addition, a number of lesions secondary to kidney malfunction were observed in the treatment groups, such as parathyroid hyperplasia, osteitis fibrosa in the femur and periarteritis in several organs.

Dose-related increases in hyperplasia and hyperkeratosis of the oesophagus and of the squamous mucosa of the forestomach were observed in all treatment groups. In the forestomach and glandular stomach of treated animals, increased incidences of foci of necrosis and ulceration were also found. In the duodenum, mucosal hypertrophy was observed. Furthermore, mucosal hypertrophy occurred in treated rats. The gastrointestinal lesions are considered to be the result of local irritating effects of chlorothalonil. Dose-dependent increases in renal tumours, predominantly tubular adenomas and carcinomas, were found in all treatment groups. In the forestomach of the treated groups, a dose-dependent increase in papillomas occurred, and two cases of squamous carcinomas were observed at the high dose. A few anaplastic renal carcinomas and transitional cell adenomas, noted at the middle and high doses, may not have been treatment related. A dose-related decrease in the incidence of mononuclear cell leukaemia was found.

In the present study, the main target organ for systemic toxicity was the kidney. The LOAEL was 40 mg/kg bw per day, the lowest dose tested, based on macroscopic and histopathological lesions of the kidney, increased incidence of kidney tumours, changes in urinalysis parameters and increased kidney weights. The LOAEL for local gastrointestinal effects was 40 mg/kg bw per day, the lowest dose tested, based on histological changes in the oesophagus, forestomach and glandular stomach and duodenum and an increased incidence of forestomach papillomas (Wilson & Killeen, 1985b, 1986c).

In a combined chronic toxicity and carcinogenicity feeding study, chlorothalonil (purity 98.3%) was administered in the diet to Fischer 344 rats (65 rats of each sex per dose). Concentrations of chlorothalonil in the diet were adjusted based on body weight and food consumption data in order to achieve the desired intake of 0, 1.8, 3.8, 15 or 175 mg/kg bw per day. Treatment duration was approximately 112 weeks in males and 124 weeks in females, except for males of the high dose group, which were killed during week 99 due to increased mortality. The animals were checked twice daily for mortality and clinical signs. Body weight and food consumption were measured weekly for the first 14 weeks and once every 2 weeks thereafter. Ophthalmoscopy was performed pretest and during month 23. For haematological and clinical chemistry examinations, blood samples were collected from 10 animals of each sex per group after 12 (haematology only), 18 and 24 months and at termination. Urinalysis was performed at 10, 11, 18 and 24 months and at termination. Pooled urine samples were collected at 11, 14, 18 and 25 months to investigate the cause of dark-coloured urine observed in the high dose group. At 12 months, an interim necropsy was performed on 10 animals of each sex per dose. During interim and terminal necropsies, all animals were examined macroscopically. Brain, liver and kidneys were weighed. Kidneys, stomach and renal and mesenteric lymph nodes were histologically examined. Statements of adherence to QA and GLP were included.

Increased mortality was observed in males of the high dose group. From 5 months onwards, urine of the high-dose animals was darker yellow than that of control animals. Statistically significant reductions in body weight gain (up to 27%) were observed in the high-dose animals throughout most of the study. Mild (up to 8%) but statistically significant reductions in body weight gain were also observed in males of the 15 mg/kg bw per day group.

Food consumption was not affected by treatment, but food conversion efficiency (body weight gain per unit food consumption) was decreased in high-dose animals during the first 8 weeks of treatment. No treatment-related effects on ophthalmoscopy or haematology were found. Statistically

significant increases in serum BUN levels in males of the 15 mg/kg bw per day group (up to 38%) and high dose group (up to 141%) and in creatinine levels in high-dose males (up to 157%) were observed. Serum albumin levels were decreased in high-dose males (up to 16%). Statistically significant reductions in ALAT in animals at the high dose and in females at 15 mg/kg bw per day and reduced levels of AP in high-dose males were considered not adverse. Cholesterol levels were statistically significantly increased (55%) in high-dose animals. Urinalysis showed an increased volume and decreased specific gravity in high-dose males.

At 12 months, absolute (up to 26%) and relative (up to 46%) kidney weights were increased in both sexes at the high dose. Although statistically significant, increases in relative kidney weight in males at 3.8 and 15 mg/kg bw per day were relatively small (≤9%). At termination, increases in absolute and relative kidney weights were found in males at 15 mg/kg bw per day (17%) and in both sexes at the high dose (up to 63%). Macroscopic examination revealed an increase in incidence of the granularity of the kidney surface and cortical cysts in the high-dose animals. In high-dose males, an increased incidence of pelvic epithelial hyperplasia was observed. In both sexes at the high dose, increased incidences of forestomach abnormalities and parathyroid enlargement were noted. Histopathological examination revealed dose-dependent increases in incidence and severity of focal tubular epithelial hyperplasia in males at 15 mg/kg bw per day and higher and in females at 3.8 mg/kg bw per day and higher. Increased incidences and severity of clear cell hyperplasia in the kidney were found in females of the 15 mg/kg bw per day group and in both sexes of the high-dose group. Increased incidences of hyperplasia, hyperkeratosis and erosions/ulcers were noted in the forestomachs of animals dosed at 3.8 mg/kg bw per day and higher. Inclusion cysts in the forestomach were found in high-dose females. Erosions of the glandular stomach were observed in both sexes of the high dose group. In males of the 15 mg/kg bw per day group and in both sexes at the high dose, increased incidences of tubular adenomas and carcinomas were found. Papillomas of the squamous mucosa were found at doses of 3.8 mg/kg bw per day and higher.

The NOAEL for systemic toxicity was 1.8 mg/kg per day, based on an increased incidence of tubular epithelial hyperplasia in females at 3.8 mg/kg bw per day. The NOAEL for local effects in the forestomach was 1.8 mg/kg bw per day, based on the observation of hyperplasia, chronic irritation and papillomas at 3.8 mg/kg bw per day (Wilson & Killeen, 1989).

In a 2-year feeding study, chlorothalonil (purity 99.28%; batch No. NF 28/01) was administered in the diet to Crl:CD (SD) BR rats (70 rats of each sex per dose) at 0, 15, 60, 240 or 1200 ppm, equal to 0, 0.7, 2.7, 10.6 and 54 mg/kg bw per day for males and 0, 0.9, 3.3, 13.9 and 70 mg/kg bw per day for females, respectively. The animals were observed at least once per day for clinical signs. A detailed palpation was performed daily for the first 4 weeks and weekly thereafter. Body weight was measured weekly, and food consumption was measured twice per week. Water consumption in the satellite animals was measured during weeks 12, 25 and 51. Ophthalmoscopy was performed on all animals prior to treatment and in control and high-dose animals in weeks 52 and 104. For haematological and clinical chemistry examinations and urinalysis, blood and urine samples were collected from 10 animals of each sex per group during weeks 13, 26, 52, 54 (blood samples only) and 78 and at termination. Satellite groups of 20 rats of each sex per dose were killed for interim examination at 52 weeks. All rats were subjected to gross pathology, and selected organs were weighed. Non-protein thiol concentrations in fresh liver samples were measured. A wide range of tissues was evaluated microscopically. Statements of adherence to QA and GLP were included.

Mortality was not affected by treatment. In the high dose group, generalized yellow staining of the fur was observed from week 16 onwards. Ophthalmoscopy revealed no treatment-related effects. Body weight gains were reduced in high-dose females during several treatment periods. During weeks 1–4, body weight gains were reduced in all female treatment groups. However, no effects on final body weights were observed in any treatment group. Mean red cell values (packed cell volume, haemoglobin

or red blood cell count) were increased in high-dose males and to a lesser extent in males treated with 240 ppm. Occasionally, mean cell haematocrit values were also lower in these animals. ALAT activity was lower at 240 and 1200 ppm. ALAT activity could be restored by adding pyridoxal-5′-phosphate (PLP), a cofactor required for expression of ALAT, to the incubate. Thus, the decreased ALAT activity was ascribed to a deficiency in PLP due to chlorothalonil metabolism. No other changes related to PLP deficiency (e.g. lymphopenia and effects on skin and general condition) were observed. It was therefore concluded that the decrease in ALAT activity is not toxicologically relevant. From week 52, statistically significantly higher urinary protein concentrations were found in males treated with 1200 ppm.

Effects on kidney weights and macroscopic and histological findings are presented in Table 2.

Effects of chlorothalonil treatment on kidney weight and kidney macroscopy and histology were observed, although effects did not always occur in a dose-dependent manner. Taking all effects on the kidney into account, it is concluded that adverse kidney effects occurred at dietary doses of 240 and 1200 ppm.

The NOAEL for systemic effects was 60 ppm, equal to 2.7 mg/kg bw per day, based on haematological changes, increases in kidney weight and changes in kidney macroscopy and histology. The NOAEL for local effects was 15 ppm, equal to 0.7 mg/kg bw per day, based on macroscopic and histological effects observed in the stomach.

The increased incidence of tumours in the forestomach (squamous cell papilloma and carcinomas) at 1200 ppm was attributed to chronic irritation of the non-glandular region of the stomach by chlorothalonil (Spencer-Briggs et al., 1996).

2.4 Reproductive toxicity

(a) Multigeneration studies

Rats

In a dietary two-generation study of reproductive toxicity performed in accordance with OECD test guideline 416, chlorothalonil (purity 98.1%) was administered to Sprague-Dawley (CD-VAF) rats (35 of each sex per dose for both the F_0 and F_1 generations). Concentrations were 0, 500, 1500 and 3000 ppm. Based on the lowest weekly compound intake, this is equal to 0, 22.6, 68 and 145 mg/kg bw per day in males and 0, 30.9, 94 and 201 mg/kg bw per day in females of the F_0 generation and 0, 21.7, 68 and 138 mg/kg bw per day in males and 0, 31.5, 95 and 196 mg/kg bw per day in females of the F_1 generation. The rats were observed at least once per day for clinical signs. Detailed physical examinations were performed weekly for all males throughout the study and for all females prior to mating and during the 2 resting weeks in between weaning of the F_{1a} generation and mating for the F_{1b} generation. Body weight and food consumption were recorded weekly in males, except during the mating periods. In females, body weight and food consumption were measured weekly prior to mating and during the resting periods. Body weights were also recorded on gestation days (GDs) 0, 7, 14 and 21 and lactation days (LDs) 0, 7, 14 and 21 for the F_0 and F_1 females. On postnatal day (PND) 4, litters were culled to eight pups. Until weaning at PND 21, litters were examined for numbers of live and dead pups, litter weight, pup weight and sex, clinical signs and external alterations. After the F_{1b} or F_{2b} litter production, all F_0 and F_1 parents were subjected to gross pathology, and reproductive organs, brain, liver, spleen, adrenals, pituitary and kidneys were weighed. The reproductive organs, kidneys, stomachs and gross lesions of all F_0 and F_1 parents of the control and high dose groups were histologically examined. In addition, kidneys, stomachs and gross lesions of parental animals of the low and middle dose groups, the epididymis, prostate and seminal vesicles of the F_0 adults and the testes of the F_1 adults were examined. Statements of adherence to QA and GLP were included.

There were no treatment-related effects on mortality, clinical signs or reproductive parameters. At termination, body weight gains in F_0 and F_1 adults were decreased at the middle (F_0 males only) and

Table 2. Effects on kidney weight and macroscopic and histological findings in rats given chlorothalonil in feed for 2 years[a]

	Dietary concentration (ppm)									
	0		15		60		240		1200	
	M	F	M	F	M	F	M	F	M	F
Relative kidney weight (% of control)										
Interim kill	—	—	104	99	109*	101	117*	101	128*	113*
Terminal kill	—	—	99	109	88	112	101	110	121*	107
Macroscopy										
Non-glandular stomach (terminal kill)										
Depressions	8	6	8	6	21	21	30	32	31	34
Thickening	2	4	6	0	6	7	20	18	30	35
White	8	6	8	6	21	21	30	32	31	34
Limiting ridge prominent	0	0	0	0	0	0	1	0	6	4
Kidney										
Enlargement	10	5	13	4	6	2	11	1	22	5
Uniform cortical scarring	7	10	10	6	7	4	11	3	22	7
Histology										
Non-neoplastic findings										
Stomach (non-glandular region)										
- Epithelial hyperplasia and hyperkeratosis	6	6	10	13	21	26	45	46	50	49
- Ulceration	3	0	4	2	8	11	15	10	7	20
- Submucosal fibrosis and inflammatory cells	2	3	5	0	7	11	13	9	8	14
Stomach (limiting ridge)										
- Epithelial hyperplasia and hyperkeratosis	2	2	0	2	4	3	7	4	19	11
Kidney										
- Dilated basophilic cortical tubules (interim kill)	2	5	4	3	11	8	8	11	10	9
- Progressive glomerulonephrosis (terminal kill)	26	13	30	14	22	15	30	18	35	20
Neoplastic findings										
Stomach (non-glandular region)										
- Squamous cell carcinoma	0	0	0	0	0	0	0	1	2	0
- Squamous cell papilloma	0	0	0	0	0	0	0	1	1	1

* $P < 0.05$

F, female; M, male

[a] Numbers for macroscopy and histology represent incidence out of 50 animals of each sex per dose examined.

high doses (both sexes in F_0 and F_1 generations). In low-dose F_1 males, a reduced body weight gain during the first 2 weeks of treatment was observed; however, no effects were observed in the mid-dose F_1 males. In low-dose F_1 females, a small (9%) but statistically significant reduction in body weight gain was observed over the first 14 weeks of treatment only. Food consumption in the high-dose F_0 animals was reduced during week 1; thereafter, relative food consumption was increased for most weeks in these rats. The high-dose F_1 females and mid- and high-dose F_1 males showed an increase in relative food consumption. In the offspring, no treatment-related gross malformations and no effects on the number of live or stillborn pups, pup sex ratio, pup survival or physical condition of the pups during lactation were observed. At the high dose, mean pup weights at LD 21 were reduced in pups

from all four matings: F_{1a}, F_{1b}, F_{2a} and F_{2b}. Occasionally, small (8%) but statistically significant reductions in pup body weight were observed in one of the four matings at the low and middle doses. In all treatment groups, low incidences of enlarged or slightly green kidneys and kidneys with granular capsular surface were found. Furthermore, at all dose levels, there was an increase in stomachs with thickened mucosa and foci. Low incidences of enlarged cervical lymph nodes were noted at the middle and high doses. Histological examination of the kidneys revealed increased incidences of tubular epithelial hyperplasia, tubular hypertrophy, clear cell hyperplasia, pigmentation and karyomegaly in males of all doses and in females of the middle and high doses. Incidences were higher in males than in females. In addition, in high-dose males, one tubular adenoma and one tubular carcinoma were observed. In all treatment groups, increased incidences of hyperkeratosis and squamous cell hyperplasia of the forestomach were found.

The LOAEL for parental toxicity was 500 ppm, equal to 21.7 mg/kg bw per day, the lowest dose tested, based on the macroscopic and microscopic effects on kidneys and forestomach in both sexes observed at all doses. The NOAEL for offspring toxicity was 1500 ppm, equal to 68 mg/kg bw per day, on the basis of the consistent decrease in body weight of the F_1 pups at the high dose. The NOAEL for reproductive effects was 3000 ppm, equal to 138 mg/kg bw per day, the highest dose tested (Lucas & Killeen, 1990).

(b) Developmental toxicity

Rats

In a developmental toxicity study, performed in accordance with OECD test guideline 414, chlorothalonil (purity 98.0%) was administered in 0.5% aqueous methyl cellulose by oral gavage to pregnant Sprague-Dawley rats (25 per dose group) on GDs 6–15 at a dose of 0, 25, 100 or 400 mg/kg bw per day. Animals were examined twice daily for clinical signs. Body weight was recorded at GDs 0, 6, 9, 12, 15, 18 and 20. Food consumption was recorded over the intervals between body weight measurements. At termination on GD 20, numbers of live and dead fetuses and early and late resorptions were recorded, live fetuses were weighed and sexed, and external alterations and intrauterine location were recorded. Approximately one half of the fetuses from each litter were examined for visceral abnormalities. Also, all live fetuses with malformations during the external examination were examined for soft tissue alterations. All remaining live fetuses were examined for skeletal alterations. Dams were necropsied. Statements of adherence to QA and GLP were included.

At 100 mg/kg bw per day, one female died due to an intubation error. In the high dose group, three dams died. One or two days before they died, the animals displayed laboured breathing, brown material around the nose and mouth, reduced activity and body temperature and/or white discoloration of the faeces. The cause of death could not be established. At the high dose, mucus, loose or white discoloration of the faeces, brown material around nose and mouth, matting of the urogenital fur and a slightly increased incidence of hair loss were observed. In two dams, red vaginal discharge was found. Compared with control animals, body weight gain over the treatment period was decreased by 7%, 16% and 49% in the low, middle and high dose groups, respectively, reaching statistical significance at the high dose. Food consumption was decreased in all treatment groups during the first 3 days of treatment and throughout the treatment period in high-dose animals. No treatment-related macroscopic changes were observed in the dams. The mean numbers of corpora lutea, implantation sites, resorptions and live fetuses, fetal weights and sex ratios were comparable across all groups. In the high dose group, there was a slight increase in postimplantation loss and a slight decrease in viable litter size, which was in part attributable to one dam, with early resorption of 16 out of 17 implantation sites. There were no treatment-related fetal external, visceral or skeletal malformations or variations or adverse effects on fetal skeletal ossification observed at any dosage.

The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on increased mortality, clinical signs and reduced body weight and food consumption. The NOAEL for fetal toxicity was

100 mg/kg bw per day on the basis of the increased postimplantation loss and reduced viable litter size (Mizens, Wilson & Killeen, 1983).

Rabbits

In a developmental toxicity study performed in accordance with OECD test guideline 414, chlorothalonil (purity 98.1%) was administered in 0.5% aqueous methyl cellulose by gavage to pregnant New Zealand White rabbits (20 per dose) on GDs 7–19, at doses of 0, 5, 10 or 20 mg/kg bw per day. Animals were examined twice daily for clinical signs. On GDs 0, 7, 10, 13, 16, 19, 24 and 30, a detailed physical examination was performed, and body weight and food consumption were recorded. At termination on GD 30, numbers of live and dead fetuses, fetal resorptions and corpora lutea were recorded. Live fetuses were weighed and sexed, and external/visceral alterations and intrauterine location were recorded. All live fetuses were subsequently examined for skeletal alterations. Dams were necropsied. Statements of adherence to QA and GLP were included.

Treatment did not cause maternal mortality or clinical signs. Over the entire treatment period, the does of the high dose group showed body weight loss (−8 g versus a body weight gain of 136 g in control animals). Food consumption was significantly reduced on day 7. Premature delivery occurred in two animals of each of the control and middle dose groups and in one animal of each of the low and high dose groups. Gravid uterine weights, fetal weights, sex ratios and the numbers of litters, corpora lutea, implantation sites, resorptions, live fetuses and postimplantation losses were comparable across all groups. No effects of treatment on development of the fetuses were noted.

The NOAEL for maternal toxicity was 10 mg/kg bw per day, based on body weight loss during treatment with chlorothalonil at 20 mg/kg bw per day. The NOAEL for fetal toxicity was 20 mg/kg bw per day, the highest dose tested (Wilson & Killeen, 1988b).

2.5 Genotoxicity

The results of genotoxicity tests with chlorothalonil are summarized in Table 3.

Table 3. Results of studies on the genotoxicity of chlorothalonil

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	0.33–6.6 µg/plate (±S9)[a]	97.8	Negative	Kouri (1977a)
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	2.5–1000 µg/plate (±S9)[b]	Unknown	Negative	Haworth (1984a)
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	0.5–50 µg/plate (−S9) 0.16–16 µg/plate (+S9)[c]	Unknown	Negative	Haworth (1984b)
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	1–10 µg/plate (±S9)	99.3	Negative	Shirasu, Moriya & Watanabe (1977)
	Escherichia coli Wp2 uvrA	1–500 µg/plate (−S9)		Negative	
		2–100 µg/plate (+S9)[d]		Negative	
Reverse mutation	*Bacillus subtilis* H17 (repair competent) *B. subtilis* M45 (repair deficient by uvrB mutation)	2–200 µg/plate[e]	99.3	Negative	Shirasu, Moriya & Watanabe (1977)

Table 3 (contd)

End-point	Test object	Concentration	Purity (%)	Results	Reference
Gene mutation	V79 Balb/3T3 cells	0.3 µg/ml (−S9) 0.3–0.3 µg/ml (+S9)	97.8	Negative	Kouri & Joglekar (1977)
Chromosomal aberrations	Chinese hamster ovary cells (CHO-K1)	0.03–0.3 µg/ml (−S9) 0.6–6 µg/ml (+S9)	98.8	Positive Negative	Putman (1986)[f]
In vivo					
Micronucleus formation	Male mouse bone marrow	4–2500 mg/kg bw (twice by gavage)	98.2	Negative	Siou (1983a)
Micronucleus formation	Mouse bone marrow	Males: 1000–10 000 mg/kg bw Females: 500–5000 mg/kg bw (single dose by gavage)[g]	Unknown	Inconclusive	Siou & Lerond-Conan (1985)
Micronucleus formation	Male rat bone marrow	8–5000 mg/kg bw (twice by gavage)	98.2	Negative	Siou (1983a)
Micronucleus formation	Male Chinese hamster bone marrow	4–2500 mg/kg bw (twice by gavage)	98.2	Negative	Siou (1983a)
Chromosomal aberrations	Male mouse bone marrow	4–2500 mg/kg bw (twice by gavage)	98.2	Negative	Siou (1981)
Chromosomal aberrations	Male mouse bone marrow	250–2500 mg/kg bw (single dose by gavage)	98.2	Negative	Siou (1983b)
Chromosomal aberrations	Male rat bone marrow	8–5000 mg/kg bw (twice by gavage)	98.2	Negative	Siou (1981)
Chromosomal aberrations	Male rat bone marrow	500–5000 mg/kg bw (single dose by gavage)	98.2	Negative	Siou (1985b)
Chromosomal aberrations	Male rat bone marrow	500–2000 mg/kg bw (5 daily doses by gavage)	98.85	Negative	Kajiwara & Furusho (1994)
Chromosomal aberrations	Male Chinese hamster bone marrow	500–5000 mg/kg bw (single dose by gavage)[h]	98.2	Negative	Siou (1985b)
Chromosomal aberrations	Male Chinese hamster bone marrow	50, 125, 250 mg/kg bw (5 daily doses by gavage)[i]	98.2	Equivocal	Siou (1985a)
Chromosomal aberrations	Male Chinese hamster bone marrow	8–5000 mg/kg bw (twice by gavage)[j]	98.2	Negative	Siou (1981)
Chromosomal aberrations	Male Chinese hamster bone marrow	187.5–750 mg/kg bw (5 daily doses by gavage)	98.3	Negative	Mizens & Laveglia (1995)

S9, 9000 × *g* rat liver supernatant

[a] Based on the results of a toxicity test using TA1538, the highest dose selected for the mutagenicity test was 6.6 µg/plate.

[b] Owing to limited solubility, the highest dose was 1000 µg/plate. S9 homogenate was prepared from kidneys from Aroclor 1254–treated rats.

[c] Selected maximum dose levels were about half the dose that produced ≥90% decrease in number of revertant colonies. S9 homogenate was prepared from kidneys from Aroclor 1254–treated rats. Test was performed only once.

[d] S9 homogenate was prepared from livers from Aroclor 1254–treated rats. No justification for the chosen dose range was provided.

[e] Rec assay. End-point: Differential killing due to difference in DNA repair competence. Test was performed only once. No justification for the chosen dose range was provided.

[f] Statements of adherence to QA and GLP were included.

[g] At 48 h after dosing, statistically significant increases in micronuclei were observed in males of the 10 000 mg/kg bw group. It is noted that at this dose, animals displayed signs of severe toxicity. One male died. The dose of 10 000 mg/kg bw is 5 times higher than the limit dose recommended by OECD.

[h] Oral administration of chlorothalonil at 2500 mg/kg bw per day to hamsters caused a statistically significant ($P < 0.05$) increase in the incidence of mitotic figures with anomalies at the 6 h time point. However, statistical significance was achieved only when gaps are included in the analysis. No statistically significant difference in chromosomal breaks, excluding gaps, was observed between treatment and control groups.

[i] At 50 and 250 mg/kg bw, the number of mitotic cells with breaks (excluding gaps) was increased 3- and 4-fold, respectively, compared with controls. Statistical significance was not reached. At 125 mg/kg bw, no increase was observed.

[j] A statistically significant, but weak, increase in chromosomal gaps was observed at the high dose. At this dose, 4 out of 13 animals died. No chromosomal breaks, excluding gaps, were observed.

An Ames test by Kouri (1977b) was considered not suitable for evaluation owing to several deficiencies.

2.6 Special studies

(a) Effects on kidneys

In a study to investigate the early morphological changes in the kidney induced by chlorothalonil, the substance (purity 97.9%) was administered to groups of male Fischer 344 rats (three per dose) in the diet at 1750 ppm (equal to about 223 mg/kg bw per day) for 4 days or by gavage for 1, 2, 3 or 4 days at a dose of 175 mg/kg bw per day. Control rats received vehicle (0.5% aqueous methyl cellulose) for 4 days. The animals dosed by gavage were killed 24 h after their last dose. Animals fed test diet were killed after 4 days of treatment. The kidneys from all animals were examined microscopically. Statements of adherence to QA and GLP were included.

Animals given chlorothalonil in the diet for 4 days showed widespread vacuolar degeneration of the proximal tubular epithelium. The same lesions were observed in animals given chlorothalonil by gavage for 2 days or more. More tubuli were affected in the animals that received the test substance via the diet than in the animals receiving the test substance by gavage (Ford & Killeen, 1988).

Chlorothalonil (purity 99.6%; batch No. ASJ10125-03S) was administered to groups of five male Fischer 344 rats by gavage in a 0.5% aqueous methyl cellulose suspension at 175 mg/kg bw per day for 1, 2, 3 or 4 days. A group of control animals was dosed with vehicle only for 4 days. Animals were checked daily for clinical signs. Body weights were determined before treatment started and at day 5, prior to termination. Food consumption was measured daily. Animals were killed 24 h after the last day of treatment. Kidneys from all animals were weighed and examined microscopically.

There were no treatment-related effects on clinical observations, body weights or food consumption. Relative kidney weights were slightly but statistically significantly increased (9%) in the animals dosed for 4 days. Kidney weights in the animals dosed for 1, 2 or 3 days were similar to control values. Daily treatment with chlorothalonil induced a time-dependent effect on the S2 portion of the proximal convoluted tubules of the kidney. The earliest appearance of the effect was seen after 1 day of dosing (the earliest time point sampled) when single hypereosinophilic cells were observed in the S2 region of the proximal convoluted tubules. This progressed into a hydropic change affecting the whole S2 tubulus after 2, 3 or 4 days. The severity of the finding of eosinophilic cells gradually decreased with repeated dosing, whereas the severity of hydropic change increased. In addition, increased numbers of mitotic cells were present in the S2 proximal tubules after 2–4 days of treatment (Rattray, 2000).

Chlorothalonil (purity 97.9%) was administered by gavage to three groups of male Fischer 344 rats (five per group) in a total daily dose of 175 mg/kg bw, administered in two equal doses given 8 h apart. Group I rats (controls) received 0.5% aqueous methyl cellulose and were killed 16 h after the last administration. Groups II and III rats received chlorothalonil for 1 day only. Group II rats were killed 4 h and group III rats 16 h after the last dose. Group IV rats were similarly dosed on 2 consecutive days and were killed 16 h after the last dose. The kidneys of all animals were examined by light and electron microscopy. The eyes of animals from groups II and IV were examined microscopically. Statements of adherence to QA and GLP were included.

Body weights were reduced in group IV rats. Slightly soft stools were observed in groups III and IV. Light microscopic examination revealed tubular epithelial vacuolation in groups III and IV. Electron microscopy showed focal epithelial swelling and stippled cytoplasmic vacuoles in the

proximal convoluted tubules of the kidneys in some animals of group II and in all animals of groups III and IV. The incidence and severity of these findings were higher in animals treated for 2 days with chlorothalonil (group IV) than in animals treated for 1 day, and effects were more severe 16 h after (group III) than 4 h after the last dose (group II). In addition, in group IV, coalescence of small vacuoles and multiple stippled large vacuoles were observed. No changes were observed in the eyes (Killeen & Laveglia, 1993).

Chlorothalonil (purity 97.9%) was administered to groups (n = 3) of male Fischer 344 rats either in the diet for 2 or 4 days at 1750 ppm (equal to 193 and 182 mg/kg bw per day, respectively) or twice a day by gavage (doses spaced 8 h apart, actual total dose 179 mg/kg bw per day) for 2 days. The vehicle for gavage dosing was 0.5% aqueous methyl cellulose. A dietary control group was kept for 96 h; the gavage vehicle-treated control group was kept for 48 h. Gavage-dosed animals were killed after 2 days, and dietary groups were killed after 2 or 4 days. The kidneys of all animals were examined microscopically. Statements of adherence to QA and GLP were included.

In the animals treated with chlorothalonil by gavage, soft stool, reduced faeces, laboured breathing and red nasal discharge were noted. In these animals, reductions in body weight (15%) and food consumption (40%) were observed. In animals exposed to chlorothalonil through the diet, body weight gain was slightly reduced. In all chlorothalonil-treated animals, microscopic examination of the kidneys revealed tubular epithelial vacuolation, nuclear pyknosis, loss of brush border of the proximal epithelial cells and tubular epithelial degeneration (Wilson & Killeen, 1988b).

Groups of male Fischer 344 rats (four per group) received a single gavage dose of chlorothalonil (purity 99.6%; batch No. 036) at 1000 mg/kg bw. Two control groups (four males per group) received vehicle only (1% carboxymethyl cellulose). Urine was collected for 48 h over 12 h intervals. Treatment groups were killed at 24, 48 or 96 h. Control groups were killed at 24 or 48 h. At termination, blood was collected for clinical chemistry. Kidneys were histologically examined, and kidney thiol levels were determined. Statements of adherence to QA and GLP were included.

Chlorothalonil treatment induced statistically significant reductions in ALAT and AP (at 24 and 48 h) and ASAT and creatine kinase (at 24 h). Thiol analysis showed that reduced glutathione and cysteine concentrations in the kidney were elevated at 48 h. In urine, concentrations of glucose and protein and *N*-acetyl glucosaminidase activity level were 2–3 times higher than in controls at 24 and 48 h. In the S2 region of the proximal convoluted tubules of the kidneys of chlorothalonil-treated rats, time-dependent increases in eosinophilic cells, vacuolation, tubular cell necrosis and increased mitosis were found. Body and kidney weights were not affected by treatment. As plasma levels of creatinine and urea were normal and urine volume and urinary excretion of gamma-glutamyltranspeptidase activity were not increased, the study authors concluded that acute administration of chlorothalonil induces mild renal tubular injury (Cuff & Kenna, 2001).

Groups of male Fischer 344 rats (five per group) received a single gavage dose of 1000 mg/kg bw of chlorothalonil (purity 99.6%; batch No. 036). Two control groups (five males per group) received vehicle only (1% carboxymethyl cellulose). Urine was collected for 144 h over 24 h intervals for urinalysis and nuclear magnetic resonance (NMR) analysis. Treatment and control groups were killed at 96 or 144 h after dosing. At termination, blood was collected for plasma clinical chemistry. Kidneys were histologically examined. Statements of adherence to QA and GLP were included.

Body and kidney weights were not affected by treatment. In urine of treated rats, concentrations of glucose and protein and *N*-acetyl glucosaminidase and gamma-glutamyltranspeptidase activity levels were 2–3 times higher than in controls at 96 and 144 h. In addition, NMR analysis of urine showed alterations in excretion of endogenous metabolites (e.g. increased acetate and lactic acid excretion,

decreased citrate, succinate and α-ketoglutarate excretion), consistent with mild toxicity to renal tubules. Plasma clinical chemistry parameters were not affected at 96 and 144 h. At 96 h, moderate to marked vacuolation in the S2 region of the proximal tubules of the kidneys was observed in treated rats. At 144 h, there was evidence of recovery and regeneration (Kenna, Cuff & Williams, 2001).

Chlorothalonil (purity 99%; batch No. GC30M2 (I-680)) was administered in the diet to four groups of male Fischer 344 rats (six per group) for up to 28 days. Concentrations of chlorothalonil in the diet were adjusted based on body weight and food consumption data in order to achieve the desired intake of 0, 1.5, 15 or 175 mg/kg bw per day. After 7, 14, 21 and 28 days, predesignated animals were necropsied. Kidney, stomach, duodenum and abnormal tissues were histologically examined. In addition to routine histological staining, proliferating cell nuclear antigen (PCNA) staining was performed on kidneys and BrdU staining was performed on stomachs, as markers for hyperplastic activity. Duodenums were used as negative control. Statements of adherence to QA and GLP were included.

Treatment had no effect on clinical signs, body weight or food consumption. Kidney and stomach weights were increased at the middle and high doses. Macroscopic examination showed white coloration of the stomach at the high dose. Vacuolation of the epithelium of the proximal tubules of the kidneys was seen in one rat of the middle dose group at day 28 and in all animals of the high dose group at all time points. A dose-dependent increased PCNA staining in the kidneys was found in the middle dose groups at days 7, 14 and 21 and in the high dose group at all time points.

In the forestomach, oedema, erosion, inflammatory cell infiltration, increased cornification and hyperplasia of the squamous cell epithelium were observed at the middle dose on days 21 and 28 and at all time points in the high dose group. BrdU staining was dose-dependently increased in the middle dose group at days 21 and 28 and in the high dose group at all time points (Hironaka, 1996).

In a study aimed at investigating kidney cell proliferation after chlorothalonil treatment using BrdU labelling, male Fischer 344 rats were fed diets containing chlorothalonil (purity 97.9%; batch No. 1009) for up to 90 days. Concentrations of chlorothalonil in the diet were adjusted based on body weight and food consumption data in order to achieve the desired intake of 0 or 175 mg/kg bw per day. Animals were checked twice daily for clinical signs. Body weight and food consumption were measured weekly. Seven rats per dose were implanted with osmotic pumps containing BrdU 3.5 days before they were killed at day 7, 28 or 91. Animals were necropsied, and brain and kidneys were weighed. The kidneys were examined for cell proliferation. The duodenum served as control tissue. Kidneys and stomachs were also examined microscopically. Statements of adherence to QA and GLP were included.

From day 54, dark yellow urine was observed in the treatment group. During the first week, the chlorothalonil-treated rats lost weight, whereas controls gained weight. Although from week 2 onwards the weekly body weight gain was slightly higher in the treatment group than in controls, at termination in week 13, the mean body weight of the treatment group was still 4% lower than that of control animals. Total gain in body weight over the 13-week treatment period was 23% lower than in controls. Food consumption reflected the effects on body weight. A small (3%) but statistically significant reduction in brain weight of the treated animals at day 28 was considered a spurious finding. Absolute and relative kidney weights and cell proliferation in the kidneys were significantly higher in treated rats at all time points. The extent of the cell proliferation was highest at day 7 and decreased with time over the next two time points. Histological examination of the kidneys showed degeneration of the proximal convoluted tubules characterized by cytoplasmic vacuolation, nuclear pyknosis, karyolysis and cellular swelling. Some cells appeared necrotic, and tubular hyperplasia and hypertrophy were observed. In all treated animals, a thickened forestomach and erosions in the

forestomach were noted. Histological evaluation showed submucosal oedema, hyperkeratosis and squamous epithelial hyperplasia. At day 7, ulcers were noted in 4 out of 14 animals (Mizens, 1997).

Male Fischer 344 rats were dosed orally with chlorothalonil (purity not reported) at 175 mg/kg bw per day for up to 13 weeks. It was not reported whether administration was dietary or by gavage. Animals were killed at days 4 and 7 and at weeks 2, 4, 6, 8, 10, 12 and 13. Kidneys were examined histologically.

Administration of chlorothalonil caused kidney toxicity, which was evident as vacuolar degeneration of the proximal tubule seen after 4 days of dosing with progression to foci of basophilic tubules and/or proliferative interstitial fibrosis seen from 2 weeks onwards (Mercier, 1986).

Male Fischer 344 rats (15 per group) were treated daily by gavage with 0 or 150 mg/kg bw per day of monoglutathione conjugate of chlorothalonil (purity 92.5%; batch No. SDS-66382) or an equimolar dose (i.e. 75 mg/kg bw per day) of chlorothalonil (purity 97.9%; batch No. SDS-2787-0901) for approximately 93 days. The vehicle was 0.5% aqueous methyl cellulose. The animals were observed twice daily for clinical signs. Body weight and food consumption were recorded weekly. Blood and urine samples were taken for haematology, clinical chemistry and urinalysis at week 7 and at termination. Additional 24 h urine samples were taken from 10 randomly selected rats from each group on days 1 and 4 and at the end of weeks 2, 4, 8 and 12, to identify and quantify the thiol metabolites. At termination, all animals were macroscopically examined. Weights of brain, liver and kidneys were recorded, and histopathological investigations were carried out on kidneys and stomachs of all animals. Microscopy of the left and right kidneys was performed by two independent laboratories using two different fixation and staining procedures. Statements of adherence to QA and GLP were included.

In rats treated with chlorothalonil, dark yellow–coloured urine and rales were observed. In this treatment group, significantly lower body weights (5–9%) were also observed in the chlorothalonil-treated rats from week 4 onwards. Food consumption was not affected. In both treatment groups, serum ALAT was reduced. Decreases in urea nitrogen and glucose levels were statistically significant but were within normal historical ranges. In both groups, absolute and relative kidney weights were statistically significantly increased. An increase in relative brain weight in the chlorothalonil group could be accounted for by the decreases in body weight. Mucosal thickening and/or ulceration of the non-glandular stomach were seen in 13 out of 14 chlorothalonil-treated rats, but not in the other two groups. Histopathology revealed proximal tubular epithelial hyperplasia, tubular dilatation (hypertrophy), vacuolar degeneration and interstitial fibrosis in the kidney of animals in both treatment groups. In the chlorothalonil group, hyperplasia of the squamous epithelium, hyperkeratosis and occasional erosion or ulceration of the epithelium in the non-glandular portion of the stomach were observed. Such effects were not found in the group treated with monoglutathione chlorothalonil.

It appears that addition of the glutathione residue to chlorothalonil eliminates its irritation properties within the stomach. Both test substances produced equivalent effects in kidneys (increased kidney weight and histopathological changes) (Ford & Killeen, 1987).

(b) Proposal for a mode of action for renal tumour formation

The mode of action for renal tumour formation using the structure of the International Programme on Chemical Safety (IPCS) conceptual framework for cancer risk assessment is discussed in Appendix 1.

3. Studies with the metabolite SDS-3701

4-Hydroxy-2,5,6-trichloroisophthalonitrile (company code: SDS-3701) is a soil and plant metabolite of chlorothalonil and has also been identified as a metabolite in ruminants. The toxicology of this metabolite was extensively tested.

Table 4. Results of studies of acute toxicity with SDS-3701, a chlorothalonil metabolite

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	Reference
Rat	Carworth	Male	Oral	Unknown	332	Wazeter (1971)[a]
Rat	Sprague-Dawley	Male	Oral	98	422	Hastings (1973)[b]
		Female			242	

[a] No mortality was observed at doses up to and including 147 mg/kg bw. The lowest dose producing mortality was 215 mg/kg bw.
[b] Death was preceded by a typical pattern of tremors, extreme jerks on stimulation and some salivation, followed by ataxia and terminal convulsions, with a number of cases of nasal haemorrhage and cyanosis. No mortality was observed at doses up to and including 100 mg/kg bw. The lowest dose producing mortality was 150 mg/kg bw.

3.1 Biochemical aspects of the metabolite SDS-3701

(a) Absorption, distribution and excretion

The toxicokinetics of a single oral administration of ^{14}C-ring-labelled SDS-3701 (radiochemical purity 97%; chemical purity 99%) at a dose of 4.3 or 62.4 mg/kg bw was studied in groups of four male Sprague-Dawley rats. The vehicle was polyethylene glycol 400. Urine and faeces were collected at 24 h intervals until termination at 96 h post-dose. Selected tissues were collected at termination. Samples were analysed for radioactivity directly by liquid scintillation counting or by combustion liquid scintillation counting.

About 100% of administered radioactivity was recovered at both dose levels. In the low and high dose groups, 74% and 65%, respectively, of the administered dose were recovered in faeces, whereas urine contained 7.5% and 9.7%, respectively. After 4 days, the remainder of the radiolabel was recovered from body tissues. At the low and high doses, respectively, radiolabel was recovered in blood (6.9% and 5.0%), muscle (7.9% and 4.7%), fat (3.1% and 3.6%), liver (2.1% and 1.2%) and kidneys (0.73% and 0.37%). No other analysed tissue, other than the gastrointestinal tract, contained more than 0.5% of radiolabel. Highest concentrations (microgram equivalents per gram) of radiolabel were found in liver. The tissue and urine levels indicate an oral absorption of at least 26–30% of the administered dose. Biliary excretion was not measured, so actual oral absorption may be higher (Jarrett, Stallard & Bachard, 1978).

3.2 Toxicological studies on the metabolite SDS-3701

(a) Acute toxicity

Results of studies of acute toxicity with SDS-3701 are summarized in Table 4.

(b) Short-term studies of toxicity

Rats

Charles River COBS CD rats (10 of each sex per dose) were fed a diet containing SDS-3701 (purity 99.6%; batch No. 9813-30-3) for approximately 60 days. The concentrations of test compound in the diet were changed as necessary to obtain doses of 0, 10, 20, 40, 75, 125, 250, 500 and 750 mg/kg bw per day. Animals were checked daily for clinical signs. A detailed physical examination was performed once per week. Body weights and food consumption were determined weekly. Haematology, clinical chemistry and urinalysis were performed prior to termination. At termination, all animals were macroscopically examined, and selected organs were weighed. The following tissues were subjected to histological examination: adrenals, gonads, small intestine, caecum, colon, kidneys, liver, spleen, stomach, urinary bladder, all visible lesions and tissue masses. Statements of adherence to QA and GLP were included.

No mortality was observed at doses up to and including 40 mg/kg bw per day. At 75 mg/kg bw per day, three females died, whereas at higher doses, all females died. At 125, 250, 500 and 750 mg/kg bw per day, 5, 9, 9 and 10 males died, respectively. The principal alteration in these animals was paleness of the bone marrow, heart, kidneys, brain, pituitary, liver and pancreas, which was indicative of anaemia. Other alterations included gastric erosions and/or ulcerations and discoloration of urine, liver and kidney, foam in the trachea, pulmonary congestion and pulmonary oedema. Piloerection was noted at all doses. At 10 mg/kg bw per day, the incidence of piloerection was only slightly higher than in controls. At doses of 20 mg/kg bw per day and higher, pallor and hyperirritability were also observed. At doses of 40 mg/kg bw per day and higher, animals displayed inactivity. The incidence and severity of the clinical signs increased with dose. Body weight gain (dose dependent) and food consumption (not dose dependent) were decreased (up to 56% in animals that survived until termination) at doses of 40 mg/kg bw per day and higher. Animals dosed at 75 mg/kg bw per day or higher gained little or no weight over the treatment period. In males treated at 250 mg/kg bw per day and higher and in females treated at 125 mg/kg bw per day and higher, no haematology, clinical chemistry or organ weight measurements were performed owing to the high mortality in these groups. Males of the 75 and 125 mg/kg bw per day groups and females of the 20 and 75 mg/kg bw per day groups showed slight to marked degrees of anaemia. In these animals, total erythrocytic count, haematocrit, haemoglobin and one or more red cell indices (mean corpuscular volume, mean cell haemoglobin concentration, mean corpuscular haemoglobin) were reduced. The slight but statistically significant decreases (3–9%) in haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin and mean cell haemoglobin concentration in the females of the 20 mg/kg bw per day group were reported to be within the normal range. Varying degrees of increases in the peripheral nucleated erythrocytes were observed in all these groups and in males given 40 mg/kg bw per day. Prothrombin time was significantly increased in females at 75 mg/kg bw per day. At doses of 75 mg/kg bw per day and higher, slight but statistically significant reductions in total serum protein, albumin, globulins and/or albumin to globulin ratios and increases in AP, ALAT, ASAT and prothrombin time were noted, indicative of mild hepatic injury. These changes were generally associated with histopathological evidence of hepatic damage. Slight reductions in total serum protein (both sexes) and albumin (females only) observed at 40 mg/kg bw per day were not accompanied by histological evidence of hepatic injury. Elevated BUN levels in a few female rats at 75 mg/kg bw per day and in males at 125, 250 and 500 mg/kg bw per day are indicative of mild kidney damage. Urinalysis revealed no treatment-related changes. Terminal necropsy of the survivors revealed flabby hearts, thin pale blood and pale yellow-brown livers and/or kidneys in some of the treated animals at 75–500 mg/kg bw per day. These lesions were considered to be consistent with the anaemic state of these animals. Three females from the 40 mg/kg bw per day dose group had pale brown kidneys, one had a pale brown liver and one had a flabby heart, but these changes were not associated with microscopic alterations.

In view of the markedly low body weights in the treatment groups, the organ weight to brain weight ratio was considered to provide a better insight into the effects of treatment on organ weight than the absolute and relative organ weights. Test material–related decreases were observed in the organ weight to brain weight ratios of the liver, spleen, kidneys, ovaries, testes and/or the heart at 40 mg/kg bw per day and above. The numbers of surviving males at doses of 250 mg/kg bw per day and higher and surviving females at doses of 125 mg/kg bw per day and higher were insufficient to perform a statistical analysis. A wide range of histopathological alterations, such as erythroid hyperplasia or hypoplasia and granulocytic depression of the bone marrow and spleen, toxic hepatitis, nephrosis, adrenocortical necrosis, auricular thrombosis, gonadal degeneration, slight myocardial degeneration, splenic atrophy and haemosiderosis of the liver and the spleen, were found at doses of 40 mg/kg bw per day and higher. The changes observed at doses of 75 mg/kg bw per day and higher occurred in animals that experienced a marked decrease in body weight gain. At 40 mg/kg bw per day, moderate erythroid hyperplasia of the bone marrow and spleen and granulocytic depression of the bone marrow were indicative of regenerative anaemia.

The NOAEL was 10 mg/kg bw per day, based on the clinical signs and slight haematological signs of anaemia observed at 20 mg/kg bw per day (Ford & Killeen, 1982a).

Dogs

Groups of Beagle dogs (four of each sex per dose) received SDS-3701 (purity ~99%; batch No. 8180-70) in the diet at 0, 50, 100 or 200 ppm, equivalent to 0, 1.25, 2.5 and 5 mg/kg bw per day, for 90 days. The animals were checked daily for clinical signs. Body weights and food consumption were recorded weekly. Haematology, clinical chemistry and urinalysis were performed prior to treatment and at weeks 4 and 13. At termination, all animals were macroscopically examined. Selected organs were weighed. Histopathological investigations were carried out on a limited number of organs of control and high-dose animals. In animals of the low and middle doses, liver, kidneys and tissues with gross lesions were histologically examined.

All dogs of the 200 ppm group died between weeks 6 and 12 of the study. In these dogs, tarry stools, anorexia and reduced food consumption were observed. At 100 ppm, soft tarry stools were occasionally observed. Mean body weights and food consumption were not affected at 50 and 100 ppm. Changes in haematological values in the treatment groups were in general not consistent over time or sex and, according to the study author, were within the normal range (historical control ranges not shown). At 4 weeks, glucose levels were decreased in high-dose animals, reaching statistical significance in females. This is considered to be the result of the reduced food intake and catabolism of the body tissues. Otherwise, clinical chemistry and urinalysis parameters were generally not affected. No treatment-related effects on organ weights were observed at 50 and 100 ppm. Effects on organ weights at 200 ppm were attributed to the emaciated state of these animals. No histopathological changes were noted at 50 and 100 ppm. At 200 ppm, bilistasis, parenchymatous degeneration, acute diffuse necrosis and focal necrosis of the liver and renal tubular degeneration were observed, among others.

The NOAEL was 100 ppm, equivalent to 2.5 mg/kg bw per day, based on the severe toxicity observed at 200 ppm (Bundy, 1975).

Beagle dogs (six of each sex per dose) received R182281 (SDS-3701; purity 96.6%; batch No. 0401) in the diet at 0, 30, 60 or 120 ppm for 52 weeks, equal to 0, 0.83, 1.8 and 3.3 mg/kg bw per day in males and 0, 0.95, 1.9 and 3.4 mg/kg bw per day in females. The dogs were checked daily for clinical signs. Detailed physical examination and body weight measurements were carried out weekly. Food consumption was determined daily. Ophthalmoscopy was performed prior to treatment and at termination. Haematology, clinical chemistry and urinalysis were carried out prior to treatment and at 3-month intervals during treatment. At termination, the animals were necropsied, and selected organs were weighed. A wide range of organs was examined histologically. Statements of adherence to QA and GLP were included.

There were no mortalities. Emaciation was observed in most males at 120 ppm. From about week 12 of treatment onwards, body weight gain was reduced (up to about 24%) in males at 120 ppm and in females at 60 and 120 ppm. Food consumption was decreased in males at 120 ppm. No treatment-related effects on ophthalmoscopic or urinalysis parameters were noted. At 120 ppm, erythrocytes, haemoglobin, haematocrit levels and platelet count were decreased, and mean corpuscular volume, mean corpuscular haemoglobin and prothrombin time were increased. In males at 60 ppm, a 12% reduction in erythrocytes was found. Dose-dependent reductions in ALAT were observed at all doses. A reduction in ALAT is not considered toxicologically relevant. At 120 ppm, significant increases were observed in serum activity of AP (up to 293%), ASAT (up to 60%) and gamma-glutamyltransferase (up to 186%) and in concentrations of glucose (up to 24%) and total bilirubin (up to 180%). The increased serum bilirubin was consistent with the haematology finding of macrocytic anaemia. In both sexes at 60 ppm, glucose levels were increased (up to 19%). Macroscopic examination revealed one male at 120 ppm with a granular, irregular surface and lighter colour of the liver and

a small testis. Absolute and relative liver weights and absolute testes weights were reduced in males at 120 ppm. Decreases in absolute thyroid and parathyroid weights were observed in both sexes at 120 ppm and in males at 30 ppm. However, as there were no correlating histological findings, these were not considered adverse. Relative kidney weights were increased in males (32%) and females (26%) at 120 ppm. Histology revealed increased incidences of hepatocyte necrosis and periportal mononuclear cell infiltrates in livers and glomerular fibrosis and cortical tubular regeneration in kidneys in both sexes at 120 ppm. The incidence of seminiferous tubule degeneration of the testes was increased in males at 120 ppm.

The NOAEL was 30 ppm, equal to 0.83 mg/kg bw per day, based on a reduction in body weight gain (females), a reduction in erythrocytes (males) and increased serum glucose levels (both sexes) (Schetter, Yoshida & Watson, 2000).

(c) Long-term studies of toxicity and carcinogenicity

Mice

In a dietary study, CD-1 mice (60 of each sex per dose) received SDS-3701 (purity 99.6%; batch No. 9813-30-3) in the food at concentrations of 0, 375, 750 or 1500 ppm, equivalent to 0, 56, 113 and 225 mg/kg bw per day, for up to 22 months. Animals were observed daily for clinical signs. Detailed physical examinations were performed weekly. Body weight and food consumption were measured weekly through week 14, biweekly through week 26 and monthly thereafter. Haematology was performed at 12 and 18 months and at termination on 10 animals of each sex per dose. At termination, all animals were subjected to gross and microscopic examination. Selected organs were weighed. Statements of adherence to QA and GLP were included.

A non-dose-dependent increase in mortality was observed in treated females. At the high dose, body weight gain was decreased in males (up to 15%) and females (up to 19%), whereas food consumption was increased up to 23% and 31% in males and females, respectively. Small (up to 6%) but statistically significant decreases in body weight gain were observed in the middle dose group, whereas food consumption was generally comparable to that of control animals. At 12 and 18 months, non-dose-dependent reductions in red blood cell counts were noted in some treatment groups. No other haematological parameters were affected. The absolute and relative liver weights of all treated male mice were statistically significantly increased compared with controls when livers with "cysts" and "visible masses" were excluded from the analyses. In females, absolute and relative liver weights were increased at the middle and high doses. Absolute but not relative kidney weights were reduced at the high dose. All macroscopic and microscopic changes were typical of those seen in mice of this age and strain. The incidences of non-neoplastic and neoplastic lesions showed no dose–response relationship.

As only a limited number of parameters were assessed, it is not considered appropriate to establish a NOAEL. In this study, SDS-3701 was not carcinogenic (Ford & Killeen, 1982b).

Rats

In a 2-year dietary study, Charles River rats (75 of each sex per dose) received SDS-3701 (purity 99%; batch No. 10495-3-17) in the food. The concentrations of SDS-3701 in the diet were changed as necessary to obtain doses of 0, 0.5, 3.0, 15/10 or 30/20 mg/kg bw per day. Owing to poor survival, decreased body weight and anaemia observed in the 15 mg/kg bw per day dose group, the dose was reduced to 10 mg/kg bw per day for all rats beginning week 30. One half of the males and all the females in the 30 mg/kg bw per day group were withdrawn from treatment during week 29 and fed control diet thereafter. The dose was reduced to 20 mg/kg bw per day for the remaining males beginning at week 30. All surviving animals in this group were necropsied after 1 year, together with 10 rats of each sex per group from all other groups.

Animals were observed daily for clinical signs. Detailed physical examinations were performed weekly. Body weight and food consumption were measured weekly through week 14 and biweekly

thereafter. Haematology, clinical chemistry and urinalysis were performed prior to treatment and at 6, 12, 18 and 24 months on 10 animals of each sex per dose. At these time points, all animals were ophthalmologically examined. Owing to the increased incidence of pale skin and/or eyes, an additional haematological examination was conducted on 10 rats of each sex from the control and high dose groups at week 21. After 1 year, 10 rats of each sex per dose were subjected to interim examination. All animals found dead or dying and all animals killed after 1 year or at termination were subjected to gross and microscopic examination. Selected organs were weighed. Statements of adherence to QA and GLP were included.

Increased mortality was observed for males in the 30 mg/kg bw per day group from week 19 onwards and in females in the same group from week 22 onwards. At week 29, mortality was about 50% in males and 75% in females of the high dose. Mortality was also increased in females at 15 mg/kg bw per day at week 29. At 30 mg/kg bw per day, pale eyes and/or skin were noted, particularly in females. Body weight gain was reduced in rats at 15/10 mg/kg bw per day (up to 19% in females only) and 30/20 mg/kg bw per day (up to 18–19% in both sexes). Food consumption relative to body weight was decreased at 30 mg/kg bw per day during the first 28 weeks of treatment. After reduction of the dose from 30 to 20 mg/kg bw per day, food consumption increased in these animals. At 15/10 and 30/20 mg/kg bw per day, decreases in red blood cell count, haematocrit, haemoglobin, mean cell volume and mean corpuscular haemoglobin were observed from week 21 onwards. Mean cell haemoglobin concentration was increased at the high dose. Females were affected to a greater extent than males. Increased numbers of reticulocytes and metarubicytes were observed at the high dose, indicative of a response of the bone marrow to anaemia. Histological examination revealed hypocellular bone marrow and increased incidence and severity of iron positive staining material in the liver at 15/10 mg/kg bw per day (females only) and 30/20 mg/kg bw per day. In animals of the interim kill, bone marrow smears indicated the presence of haemosiderin at 3 mg/kg bw per day (females only), 15/10 mg/kg bw per day and 30/20 mg/kg bw per day. At 30 mg/kg bw per day, ophthalmological examination at 6 months showed an increased incidence of pale ocular structures and spontaneous haemorrhage, probably related to the anaemic condition of the rats. The incidence of bilateral cataract disease in rats given 15/10 mg/kg bw per day was increased at 24 months. At 6 months, serum total protein, albumin, globulin and cholesterol values were significantly decreased at 30 mg/kg bw per day in both sexes and were slightly lower in females given 15 mg/kg bw per day. No treatment-related effects on urinalysis parameters were found. In rats exposed to 15 or 30 mg/kg bw per day that died before the reduction in dosage levels in week 29, macroscopic examination showed haemorrhagic areas and pale tissues. Changes in organ weight observed at 15/10 and 30/20 mg/kg bw per day generally reflected the lower body weights in these animals. An increase in relative spleen weight in females at 15/10 mg/kg bw per day may be related to the haematological changes in these animals. In high-dose animals that died before week 29, histological examination revealed a high incidence of hypocellular bone marrow and increased quantities of hepatic iron. In these animals, hepatocellular necrosis and vacuolar changes in the brain and adrenal cortex were also noted. After reduction of the dosages in week 29, bone marrow appeared normal. No treatment-related changes in the incidence of neoplastic lesions were seen.

The NOAEL was 3 mg/kg bw per day, based on increased mortality, clinical signs, reduced body weight gain, changes in haematological and clinical chemistry parameters, hypoplastic bone marrow, increased spleen weight, haemosiderin deposition in liver and bone marrow and degenerative tissue changes observed at 15/10 mg/kg bw per day (Ford et al., 1983).

(d) Reproductive toxicity

Multigeneration studies

In a dietary three-generation study of reproductive toxicity, SDS-3701 (purity >99%; batch No. 9813-30-3) was administered to Sprague-Dawley rats (15 males and 30 females per dose).

Concentrations were 0, 10, 60 and 125 ppm, equivalent to 0, 0.67, 4 and 8.3 mg/kg bw per day. Each parental generation produced two litters. In between weaning of the F_{1a} generation and mating for the F_{1b} generation, there was a 15-day rest period. The rats were observed daily for clinical signs. Detailed physical examinations were performed weekly. Body weight and food consumption were recorded weekly in males, except during the mating periods. In females, body weight and food consumption were measured weekly prior to mating and during the resting periods. Body weights of females were also recorded on GDs 0, 6, 15 and 20 and LDs 0, 4, 14 and 21. On PND 4, litters were culled to 10 pups. Until weaning at PND 21, litters were examined for numbers of live and dead pups, litter weight, pup weight and sex, clinical signs and external alterations. Parental animals were killed after LD 21 of the F_{1b} generation. All pups, except those pups from the F_{1a} generation that were selected to produce the next generation, were killed at LD 21 and macroscopically examined. All gross lesions in parental animals and offspring and an extensive range of organs of 10 animals of each sex per dose of the F_{3b} generation were histologically examined. Statements of adherence to QA and GLP were included.

There were no treatment-related effects on mortality or clinical signs. However, during the study, the presence of sialodacryoadenitis, a viral disease, was detected in animals from all groups. Statistically significant lower body weight gains (up to 17%) were occasionally observed during certain periods at 60 and 125 ppm. Food consumption was generally higher in the treatment groups than in the control animals, although no dose dependency was observed. Reproductive parameters were not affected. In the $F_{2a,b}$ and $F_{3a,b}$ litters, the number of live pups per litter was slightly reduced at 125 ppm. Pup weights at birth were comparable in all treatment groups. At LD 14 and LD 21 in all generations, lower mean pup weights were observed in pups of the middle dose (up to 18%) and high dose (up to 34%). No macroscopic or microscopic changes in parental animals or offspring were found.

The NOAEL for parental toxicity is 10 ppm, equivalent to 0.67 mg/kg bw per day, based on effects on body weight at 60 ppm. The NOAEL for offspring toxicity is 10 ppm, equivalent to 0.67 mg/kg bw per day, based on effects on body weight during lactation at 60 ppm. The NOAEL for reproductive effects is 125 ppm, equivalent to 8.3 mg/kg bw per day, the highest dose tested. The presence of sialodacryoadenitis in this test renders the scientific validity of this study questionable (Ford & Killeen, 1981).

In a dietary one-generation study of reproductive toxicity performed in accordance with OECD test guideline 415, SDS-3701 (purity 99%; batch No. 10495-23-35) was administered to Sprague-Dawley rats (12 males and 24 females per dose). Concentrations were 0, 10, 20, 30, 60 and 120 ppm, equivalent to 0, 0.67, 1.33, 2, 4 and 8 mg/kg bw per day. The parental generation produced two litters. The rats were observed twice daily for clinical signs. Detailed physical examinations were performed weekly for all males throughout the study and for all females prior to mating and during a 2-week resting period in between weaning of the F_{1a} generation and mating for the F_{1b} generation. Body weight and food consumption were recorded weekly in males, except during the mating periods. In females, body weight and food consumption were measured weekly prior to mating and during the resting periods. Body weights of females were also recorded on GDs 0, 6, 15 and 20 and LDs 0, 4, 14 and 21. On PND 4, litters were culled to 10 pups. Until weaning at PND 21, litters were examined for number of live and dead pups, litter weight, pup weight and sex, clinical signs and external alterations. Parental animals were killed after LD 21 of the F_{1b} generation. All pups were killed at LD 21. Statements of adherence to QA and GLP were included.

In the parental males of the high dose group, an increase (up to 14%) in relative food intake was consistently observed from week 6 of treatment onwards. No other treatment-related effects were observed. Reproductive parameters were not affected. In the offspring, body weights were reduced at 60 ppm (up to 14%) and 120 ppm (up to 26%). Viability index at day 4 was reduced in high-dose

pups, primarily because of the death of all pups in two litters each in the F_{1a} and the F_{1b} generations. No other treatment-related effects in the offspring were observed.

The NOAEL for the parental animals is 120 ppm, equivalent to 8 mg/kg bw per day, the highest dose tested. The NOAEL for offspring toxicity is 30 ppm, equivalent to 2 mg/kg bw per day, based on reduction in body weight at 60 ppm, equivalent to 4 mg/kg bw per day. The NOAEL for reproductive toxicity is 120 ppm, equivalent to 8 mg/kg bw per day, the highest dose tested (Bush & Ford, 1982).

Developmental toxicity

Rats. In a developmental toxicity study performed in accordance with OECD test guideline 414, SDS-3701 (purity 97.6%; batch No. 0401) was administered in 1% aqueous methyl cellulose by oral gavage to pregnant Sprague-Dawley CD rats (24 per dose group) on GDs 0–19 at a dose of 0, 5, 15 or 25 mg/kg bw per day. Animals were examined twice daily for clinical signs. Detailed physical examination and body weight measurements were performed on GDs 0, 3, 6, 9, 12, 15, 18 and 20. Food consumption was recorded over the intervals between body weight measurements. At termination on GD 20, blood from the dams was sampled for haematology. Also at GD 20, live fetuses were weighed and sexed, and the intrauterine location of fetuses was recorded. Furthermore, the numbers of live and dead fetuses, early and late resorptions, and external alterations of fetuses were recorded. The dams were macroscopically examined. Approximately one half of fetuses from each litter were examined for visceral abnormalities. All remaining live fetuses were examined for skeletal alterations. Statements of adherence to QA and GLP were included.

One rat from the middle dose group died on GD 20. In the high-dose females, there was an increased incidence of red staining in the anogenital area and/or red exudates from the vagina from GD 15 onwards. At 15 mg/kg bw per day, body weight gain over the treatment period was 64% of that of control animals. At the high dose, a body weight loss (3%) was found. Food consumption was decreased at the middle and high doses. At the middle and high doses, statistically significant reductions in mean corpuscular volume and mean corpuscular haemoglobin and increases in mean corpuscular haemoglobin concentration were observed. At the high dose, haemoglobin and haematocrit were also statistically significantly reduced. Macroscopic evaluation of the dams revealed no treatment-related effects. The numbers of early and late resorptions were slightly increased at the middle dose. The increases, although not statistically significant, were outside the historical control range. At the high dose, only one live and one dead fetus were found. At the middle dose, fetal weight was reduced by 20%. An increased incidence of 14th rudimentary ribs was found in the middle dose group. Other findings were considered not related to treatment.

The NOAEL for parental toxicity is 5 mg/kg bw per day, based on reductions in body weight gain and food consumption at 15 mg/kg bw per day. The NOAEL for fetal toxicity is 5 mg/kg bw per day, based on an increase in number of early and late resorptions, a decrease in fetal weight and an increase in the frequency of 14th rudimentary ribs at 15 mg/kg bw per day.

In this study, SDS-3701 was not teratogenic (Killeen, 1998).

Rabbits. In a developmental toxicity study, SDS-3701 (purity not reported; batch No. 8180-70) was administered in 0.5% methyl cellulose by oral gavage to pregnant Dutch Belted rabbits (10–13 per dose group) on GDs 6–18 at doses of 0, 1, 2.5 or 5 mg/kg bw per day. Animals were observed daily for clinical signs. Body weight measurements were performed on GDs 0, 6, 12, 18 and 28. At GD 28, live fetuses were weighed and sexed, and the intrauterine location of fetuses was recorded. Furthermore, the numbers of live and dead fetuses, early and late resorptions, and external alterations of fetuses were recorded. All fetuses were examined for visceral and skeletal abnormalities. The reporting of the pathology data was limited. No individual fetal data were presented. The does were macroscopically examined.

Table 5. Results of studies on the genotoxicity of SDS-3701, a chlorothalonil metabolite

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 *Escherichia coli* Wp2p, Wp2p uvrA	20–2000 µg/plate (±S9)	99.0	Negative	Kouri (1977c)
Chromosomal aberrations	Chinese hamster ovary cells (CHO-K1)	16.3–260 µg/ml (−S9) 65–520 µg/ml (+S9)	99.2	Positive	Curry (1994)[a]
Gene mutation	V79 and Balb/3T3 cells	0.3–30 µg/ml	99.0	Negative	Kouri (1977d)
In vivo					
Chromosomal aberrations	Chinese hamster bone marrow	125–500 mg/kg bw (once by gavage)	99.0	Negative	Proudlock, Taylor & Elmore (1995)[a]
Dominant lethality	Male Sprague-Dawley rats; mating schedule: two females per male	2, 4 or 8 mg/kg bw (by gavage; single or 5 days)	>98.0	Negative	Hastings (1975)
Dominant lethality	Male mice; mating schedule: two females per male	6.5 mg/kg bw (by gavage for 5 days)	>99.0	Negative	Legator (1974)

S9, 9000 × *g* rat liver supernatant

[a] Statements of adherence to GLP and QA were included.

One animal from the 2.5 mg/kg bw per day group and two animals from the 5 mg/kg bw per day group died. The causes of the deaths and the days on which the animals died were not reported. Hypothermia and hypoactivity were seen occasionally at 5 mg/kg bw per day. One female at the middle dose and four females at the high dose aborted. SDS-3701 had no effect on maternal body weight or macroscopic parameters, the number of implantation sites, the number of live or dead fetuses, live fetal weights, sex ratios or fetal anomalies. Examination of the fetuses revealed no treatment-related skeletal or visceral effects. An increase in the number of females showing early resorptions was observed at the high dose, although statistical significance was not reached.

The NOAEL for maternal toxicity was 1 mg/kg bw per day, based on a mortality and an abortion observed at 2.5 mg/kg bw per day. The NOAEL for developmental toxicity was 2.5 mg/kg bw per day, based on early postimplantation loss at 5 mg/kg bw per day.

No teratogenic effects were observed (Wazeter & Goldenthal, 1976).

(e) Genotoxicity

The results of genotoxicity studies with SDS-3701, the metabolite of chlorothalonil, are summarized in Table 5.

4. Observations in humans

No data were provided. Dermatitis due to exposure to chlorothalonil was reported in the published literature (IPCS, 1996).

Comments

Biochemical aspects

In rats given a single oral dose of chlorothalonil at 1.5–50 mg/kg bw, absorption was about 32%, with 16–22% being excreted in the bile and about 7–8% being excreted in the urine. At 200 mg/kg bw,

excretion in the bile (8%) and the urine (5%) was lower, suggesting that saturation of absorption was occurring. In females, biliary excretion was lower (–20%) and urinary excretion was higher (about +35%) than in males. Urinary excretion in mice and dogs was about 5–10% and 1.4%, respectively. In rats, the highest tissue concentrations were found in the kidney, probably due to binding to kidney proteins. Chlorothalonil is metabolized via initial glutathione conjugation and subsequent enzymatic processing of the diglutathione and triglutathione substituents via the mercapturic acid and cysteine conjugate β-lyase pathways, yielding *N*-acetyl cysteine, cysteinyl-glycine and *S*-methyl derivatives.

Toxicological data

The acute oral and dermal toxicities of chlorothalonil are low (oral and dermal LD_{50} >5000 mg/kg bw). A study of acute inhalation yielded an LC_{50} of 0.1 mg/l air. Chlorothalonil is a mild skin irritant and is severely irritating to the eye. No valid test for sensitization was available. In Environmental Health Criteria 183, it is reported that the results of studies of skin sensitization in guinea-pigs were inconclusive (IPCS, 1996).

Studies of toxicity with repeated doses showed that in mice and rats, but not in dogs, the kidney is the prime target organ for systemic toxicity attributable to chlorothalonil. In studies in mice and rats, chlorothalonil also caused local toxicity in the forestomach. In a 90-day study in mice, the NOAEL for systemic effects was 275 ppm, equal to 48 mg/kg bw per day, on the basis of an increased incidence of hyperplasia in the proximal tubules of the kidneys and increased kidney weight at 750 ppm, equal to 124 mg/kg bw per day. In a 13-week study in rats, the NOAEL for systemic effects was 10 mg/kg bw per day on the basis of increased kidney weights and hyperplasia in the kidneys at 40 mg/kg bw per day.

Studies of acute toxicity in rats have demonstrated that chlorothalonil, given by gavage, induces renal tubular necrosis in the S2 segment of the proximal convoluted tubules (hypereosinophilic cells, multifocal hydropic vacuolation). These effects were observed at doses of 175 mg/kg bw and higher. The overall NOAEL for toxic effects on the kidney in studies of acute toxicity was 60 mg/kg bw.

In a 90-day study in dogs, the NOAEL for systemic effects was 15 mg/kg bw per day on the basis of reductions in body weight gain and changes in clinical chemistry parameters (not related to kidney toxicity) at 150 mg/kg bw per day. In a 1-year study in dogs, the NOAEL was 150 mg/kg bw per day on the basis of reduced body weight gain, reduced serum albumin and total protein, and increased relative liver weight and serum cholesterol at 500 mg/kg bw per day.

In a 2-year study of carcinogenicity in mice, the LOAEL was 750 ppm, equal to 119 mg/kg bw per day (the lowest dose tested), on the basis of increased kidney weights, macroscopic changes in the kidney and forestomach, and microscopic changes in the kidney, forestomach and oesophagus. In addition, at the LOAEL, renal tubular adenomas and carcinomas in males and forestomach tumours, mainly squamous cell carcinomas, in males and females were found. In a second 2-year study of carcinogenicity in mice, no preneoplastic changes in the forestomach were observed at 10/15 ppm, equal to 1.9 mg/kg bw per day. Increased incidences of hyperplasia and hyperkeratosis of the forestomach were observed at dietary concentrations of 40 ppm, equal to 5.1 mg/kg bw per day, and higher. A slightly higher incidence of forestomach tumours was observed at a dose of 750 ppm, equal to 98 mg/kg bw per day. In this study, increased incidences of renal tubular hyperplasia and karyomegaly were observed at doses of 175 ppm, equal to 23 mg/kg bw per day, and higher. No effects on kidneys were observed at 40 ppm, equal to 5.1 mg/kg bw per day.

Three long-term studies of toxicity in rats were available. In the first study, the LOAEL was 40 mg/kg bw per day (the lowest dose tested) on the basis of macroscopic and histopathological lesions of the kidneys, an increased incidence of kidney tumours, changes in urinalysis parameters, increased kidney weights, histological changes in the oesophagus, forestomach, glandular stomach and duodenum and an increased incidence of forestomach papillomas. In a second study in rats, the

NOAEL was 1.8 mg/kg bw per day on the basis of an increased incidence of renal tubular epithelial hyperplasia in females at 3.8 mg/kg bw per day. In a third study in rats, the NOAEL was 2.7 mg/kg bw per day and the LOAEL was 10.6 mg/kg bw per day on the basis of increased kidney weight, changes in kidney macroscopy and histology, and haematological changes.

In the long-term studies of toxicity in rats, kidney tumours, predominantly tubular adenomas and carcinomas, were observed at a dietary dose equal to 15 mg/kg bw per day in males and at higher doses in males and females. The overall NOAEL for kidney tumours in rats was 3.8 mg/kg bw per day. Also in the three long-term studies of toxicity in rats, forestomach tumours (papillomas and carcinomas) were observed at doses of 3.8 mg/kg bw per day and higher.

Chlorothalonil was tested for genotoxicity in vitro and in vivo in an adequate range of studies. Chlorothalonil was not mutagenic in bacteria or in tests for gene mutation in vitro in the absence or presence of metabolic activation. The results of a test for chromosomal aberration in Chinese hamster ovary cells in vitro were positive in the absence of metabolic activation but negative in the presence of metabolic activation. However, the results of numerous tests for clastogenicity in vivo in several species (i.e. mice, rats, Chinese hamsters) given single or repeated doses were negative, except for a few inconclusive or equivocal findings.

Considering all the results of studies of genotoxicity, the Meeting concluded that it is unlikely that chlorothalonil is genotoxic.

Repeated dosing with chlorothalonil resulted in hyperplasia and tumour formation in the forestomach in rats and mice. Oral administration of a monoglutathione conjugate of chlorothalonil did not cause forestomach toxicity, suggesting that forestomach lesions are a consequence of a direct irritant effect of chlorothalonil. Chlorothalonil did not cause tumours in the oesophagus, which also has squamous epithelium. This indicates that this substance needs to be in prolonged contact with squamous epithelium in order to induce tumours. The data indicate a process that starts with irritation and cytotoxicity, followed by cell proliferation, ulceration and erosion, regenerative hyperplasia and hyperkeratosis, and ultimately resulting in forestomach tumours. Chlorothalonil did not induce tumours in the glandular stomach in rats and mice. Unlike rats and mice, humans and dogs do not have a forestomach. In a 1-year study in dogs, no stomach lesions were observed at doses up to 500 mg/kg bw per day. In a 2-year dietary study in dogs, which was evaluated by JMPR in 1992, moderate to severe gastritis was found irregularly at dietary concentrations of 15 000 ppm, equivalent to 375 mg/kg bw per day, and higher. The Meeting considered the forestomach tumours induced by chlorothalonil to be a rodent-specific lesion that is not relevant to humans, because of differences in anatomy and function.

The studies of the mode of action of chlorothalonil in kidney toxicity in rats and studies with repeated doses show that chlorothalonil-induced renal tumours occur as a direct consequence of sustained damage to the S2 segment of the proximal tubules of the kidney. The occurrence of tumours is preceded by renal cytotoxicity, which is followed by regenerative cell proliferation/hyperplasia. Renal cytotoxicity and regenerative cell proliferation occur at doses lower than or similar to those causing tumours. Cytotoxicity/regenerative proliferation is a well-established mode of action for the formation of kidney tumours, although the cause of the initial cytotoxicity may differ. On the basis of information on other chlorinated compounds, it is possible that the nephrotoxicity caused by chlorothalonil may be due to reactive metabolites formed from the renal β-lyase cleavage of cysteine *S*-conjugates transported in the renal tubular cells. This mode of action is supported by the finding that when a monoglutathione conjugate of chlorothalonil is administered orally, similar kidney lesions are observed at a comparable dose. Because β-lyase activity is lower in human kidney tissue than in that of rodents, rodents would be expected to be more sensitive to this bioactivation pathway. In a 2-year dietary study in dogs, which was evaluated by JMPR in 1992, renal glomerulosclerosis and degenerative renal tubular changes (tubular hypertrophy and dilatation) were found at dietary concentrations of 15 000 ppm, equivalent to 375 mg/kg bw per day, and higher. The kidney toxicity in dogs given high doses of chlorothalonil only is likely due to species differences in bioactivation (as

well as absorption). However, there are insufficient data on chlorothalonil to quantitatively characterize this difference in renal enzyme activity/bioactivation among rodents, dogs and humans.

The Meeting concluded that the formation of kidney tumours was the result of prolonged renal cytotoxicity and regenerative cell proliferation and is consistent with a threshold phenomenon.

In a two-generation study of reproductive toxicity with chlorothalonil in rats, the LOAEL for parental toxicity was 500 ppm, equal to 22 mg/kg bw per day (i.e. the lowest dose tested), on the basis of effects on kidneys and forestomach in males and females observed at all doses. One tubular adenoma and one tubular carcinoma were found in the kidneys of males at 145 mg/kg bw per day. The NOAEL for offspring toxicity was 1500 ppm, equal to 68 mg/kg bw per day, on the basis of a decrease in body weight of the F_1 pups at the highest dose. The NOAEL for reproductive effects was 3000 ppm, equal to 138 mg/kg bw per day, the highest dose tested.

In a study of developmental toxicity in rats, the NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of increased mortality, clinical signs, and reduced body weight and food consumption observed at 400 mg/kg bw per day. The NOAEL for fetal toxicity was 100 mg/kg bw per day on the basis of increased postimplantation loss and reduced viable litter size. In a study of developmental toxicity in rabbits, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of body weight loss during treatment with chlorothalonil at 20 mg/kg bw per day. The NOAEL for fetal toxicity was 20 mg/kg bw per day, the highest dose tested.

No data on chlorothalonil in humans were provided. In the published literature, it is reported that chlorothalonil may cause dermatitis.

Studies on the metabolite SDS-3701

4-Hydroxy-2,5,6-trichloroisophthalonitrile (company code, SDS-3701) is a soil and plant metabolite of chlorothalonil and has also been identified as a metabolite in ruminants. The toxicology of this metabolite had been tested extensively.

Biochemical aspects of the metabolite SDS-3701

After single oral doses of ^{14}C-ring-labelled SDS-3701 at 4.3 or 62.4 mg/kg bw in rats, about 65–74% and 7.5–9.7% were recovered from the faeces and urine, respectively. Radiolabel was found in the blood (5–6.9%), muscle (4.7–7.9%), fat (3.1–3.6%), liver (1–2%) and kidneys (0.4–0.7%). The highest concentrations of radiolabel were found in the liver. The tissue and urine concentrations indicate an oral absorption of at least 26–30% of the administered dose. Biliary excretion was not measured, so actual oral absorption may be higher than indicated.

Toxicological data on the metabolite SDS-3701

SDS-7301 is moderately toxic after acute oral administration (LD_{50} 242–422 mg/kg bw). Mortality was observed after single oral doses of 150 mg/kg bw or higher.

In a 2-year dietary study with SDS-3701 in mice in which a limited number of parameters were evaluated, a reduction in body weight and an increase in food consumption were observed at 1500 ppm, equivalent to 225 mg/kg bw per day. Absolute and relative liver weights were increased in females at 750 ppm, equivalent to 113 mg/kg bw per day, and higher. No treatment-related effects on the incidences of non-neoplastic and neoplastic lesions were observed at dietary concentrations up to and including 1500 ppm, equivalent to 225 mg/kg bw per day (the highest dose tested).

Dietary studies of toxicity in rats given repeated doses (60 days, 2 years) of SDS-3701 show that the haematopoietic system is the prime target organ for toxicity. The overall NOAEL in studies in rats given repeated doses of SDS-3701 was 3 mg/kg bw per day on the basis of increased mortality, clinical signs, reduced body weight gain, changes in haematological and clinical chemistry parameters, hypoplastic bone marrow, increased spleen weight, haemosiderin deposition in liver and bone marrow and degenerative tissue changes observed at 10/15 mg/kg bw per day in a 2-year dietary

study. No treatment-related changes in the incidence of neoplastic lesions were observed at doses up to and including 30/20 mg/kg bw per day.

In a 90-day study in dogs, the NOAEL was 100 ppm, equivalent to 2.5 mg/kg bw per day, on the basis of severe toxicity resulting in death observed at 200 ppm, equivalent to 5 mg/kg bw per day. In a 1-year study in dogs, the NOAEL was 30 ppm, equal to 0.83 mg/kg bw per day, on the basis of reductions in body weight gain and increased serum concentrations of glucose observed at 60 ppm, equal to 1.8 mg/kg bw per day.

SDS-3701 was tested in an adequate range of tests of genotoxicity. Most of the tests showed that SDS-3701 was not mutagenic or clastogenic. A test for chromosomal aberration in vitro in Chinese hamster ovary cells gave positive results with and without metabolic activation. However, SDS-3701 gave negative results in vivo in a test for chromosomal aberration in Chinese hamster bone marrow and in dominant lethal tests in rats and mice. The Meeting concluded that it is unlikely that SDS-3701 will show mutagenic activity in vivo.

In view of the lack of genotoxicity in vivo and the absence of carcinogenicity in mice and rats, the Meeting concluded that SDS-3701 is unlikely to pose a carcinogenic risk to humans.

In two studies of reproductive toxicity in rats, the overall NOAEL for parental toxicity was 120 ppm, equivalent to 8 mg/kg bw per day, the highest dose tested. The overall NOAEL for offspring toxicity was 30 ppm, equivalent to 2 mg/kg bw per day, on the basis of reduction in body weight at 60 ppm. The NOAEL for reproductive toxicity was 120 ppm, equivalent to 8 mg/kg bw per day, the highest dose tested.

In a study of developmental toxicity in rats, the NOAEL for maternal toxicity was 5 mg/kg bw per day on the basis of reductions in body weight gain and food consumption at 15 mg/kg bw per day. The NOAEL for fetal toxicity was 5 mg/kg bw per day on the basis of an increase in the number of early and late resorptions, a decrease in fetal weight and an increase in the frequency of 14th rudimentary ribs at 15 mg/kg bw per day. In a study of developmental toxicity in rabbits, the NOAEL for maternal toxicity was 1 mg/kg bw per day on the basis of a mortality and an abortion observed at 2.5 mg/kg bw per day. It was not reported on which day of treatment the mortality and abortion occurred. The NOAEL for developmental toxicity was 2.5 mg/kg bw per day on the basis of early postimplantation loss at 5 mg/kg bw per day. In these studies, no teratogenic effects were observed with SDS-3701.

The Meeting concluded that the existing database on chlorothalonil and its soil, plant and ruminant metabolite SDS-3701 was sufficient to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

Chlorothalonil

The Meeting established an ADI for chlorothalonil of 0–0.02 mg/kg bw based on a NOAEL of 1.8 mg/kg bw per day identified on the basis of kidney toxicity observed in long-term studies of toxicity in rats and using a safety factor of 100. This ADI provides a margin of 200 for the induction of renal tumours in rats. This ADI is similar to the one derived by JMPR in 1974 and 1990 from a 2-year study in dogs in which the NOAEL was 3 mg/kg bw per day. Previously, JMPR has based the ADI on data from dogs, arguing that the rat is particularly sensitive to kidney toxicity induced by chlorothalonil. The Meeting concluded that whereas there were some uncertainties, it was possible to establish a plausible mode of action for the renal carcinogenesis of chlorothalonil. This comprises initial conjugation with glutathione followed by sequential biotransformation to thiol derivatives in

renal proximal tubule cells by β-lyase. The thiol metabolites are cytotoxic, resulting in renal proximal tubule cell necrosis followed by regenerative proliferation. The final step is the appearance of tumours. As there are no fundamental qualitative differences between rodents and humans in the processes underlying these key events, it was not possible to dismiss human relevance on qualitative grounds. Although quantitative differences in some of the metabolic steps, such as the cysteine *S*-conjugate β-lyase pathway, have been demonstrated between rodents and humans for some other compounds sharing this mode of action, specific information on chlorothalonil was not available. Hence, the Meeting concluded that while it is plausible that humans are less sensitive to the renal effects of chlorothalonil, it was not possible to dismiss relevance to humans on quantitative grounds, nor was it possible to quantify any difference in sensitivity.

Studies of acute toxicity have demonstrated that exposure to chlorothalonil on a single day may induce kidney toxicity in rats. The overall NOAEL for kidney toxicity in studies of acute toxicity was 60 mg/kg bw. Based on this NOAEL, the Meeting established an acute reference dose (ARfD) of 0.6 mg/kg bw, using a safety factor of 100.

Given the species differences in the β-lyase bioactivation pathway, the ADI and ARfD are likely to be conservative.

SDS-3701 (4-Hydroxy-2,5,6-trichloroisophthalonitrile)

The Meeting established an ADI for SDS-3701 of 0–0.008 mg/kg bw based on a NOAEL of 0.83 mg/kg bw per day identified on the basis of a reduction in body weight gain in females, a reduction in erythrocytes in males and increased serum concentrations of glucose in males and females in a 1-year study in dogs, and using a safety factor of 100.

Levels relevant to risk assessment of chlorothalonil

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute toxicity[a]	Toxicity	60 mg/kg bw[b]	175 mg/kg bw[b]
	Two-year study of toxicity and carcinogenicity[c]	Toxicity	1.8 mg/kg bw per day	3.8 mg/kg bw per day[d]
		Carcinogenicity	3.8 mg/kg bw per day	15 mg/kg bw per day
	Two-generation study of reproductive toxicity[c]	Parental toxicity	—	500 ppm, equal to 21.7 mg/kg bw per day[d]
		Offspring toxicity	1500 ppm, equal to 68 mg/kg bw per day	3000 ppm, equal to 138 mg/kg bw per day
		Reproductive toxicity	3000 ppm, equal to 138 mg/kg bw per day[e]	—
	Developmental toxicity[a]	Maternal toxicity	100 mg/kg bw per day	400 mg/kg bw per day
		Fetotoxicity	100 mg/kg bw per day	400 mg/kg bw per day
Rabbit	Developmental toxicity[a]	Maternal toxicity	10 mg/kg bw per day	20 mg/kg bw per day
		Fetotoxicity	20 mg/kg bw per day[e]	—
Dog	Two-year study of toxicity[c,f]	Toxicity	120 ppm, equal to 3 mg/kg bw per day[e]	—

[a] Gavage administration.
[b] Overall NOAEL and LOAEL for several studies.
[c] Dietary administration.
[d] Lowest dose tested.
[e] Highest dose tested.
[f] Evaluated by JMPR in 1974 and 1992.

In a study of developmental toxicity with SDS-3701 in rabbits, early implantation loss was observed at a dose of 5 mg/kg bw per day. The NOAEL for this effect was 2.5 mg/kg bw per day. On the basis of these findings, the Meeting established an ARfD of 0.03 mg/kg bw using a safety factor of 100. The Meeting considered that the abortions and deaths observed in this study in rabbits at 2.5 and 5 mg/kg bw per day were considered to be unlikely to be induced by a single dose of SDS-3701. In studies of acute oral toxicity in rats, in which LD_{50}s of 242–422 mg/kg bw were identified, deaths were observed at doses of 150 mg/kg bw or higher. In view of information from the LD_{50} studies and the absence of other adequate data on acute toxicity, the ARfD of 0.03 mg/kg bw applies to the general population as well as women of childbearing age.

Levels relevant to risk assessment of SDS-3701

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of carcinogenicity[a]	Carcinogenicity	1500 ppm, equivalent to 225 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	3 mg/kg bw per day	10 mg/kg bw per day
		Carcinogenicity	20 mg/kg bw per day[b]	—
	One-generation study of reproductive toxicity[a]	Parental toxicity	120 ppm, equivalent to 8 mg/kg bw per day[b]	—
		Offspring toxicity	30 ppm, equivalent to 2 mg/kg bw per day	60 ppm, equivalent to 4 mg/kg bw per day
		Reproductive toxicity	120 ppm, equivalent to 8 mg/kg bw per day[b]	—
	Developmental toxicity[c]	Maternal toxicity	5 mg/kg bw per day	15 mg/kg bw per day
		Fetotoxicity	5 mg/kg bw per day	15 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	1 mg/kg bw per day	2.5 mg/kg bw per day
		Fetotoxicity	2.5 mg/kg bw per day	5 mg/kg bw per day
Dog	One-year study of toxicity[a]	Toxicity	0.83 mg/kg bw per day	1.8 mg/kg bw per day

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

Estimate of acceptable daily intake for humans

Chlorothalonil	0–0.02 mg/kg bw
SDS-3701[1]	0–0.008 mg/kg bw

Estimate of acute reference dose

Chlorothalonil	0.6 mg/kg bw
SDS-3701	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

[1] 4-Hydroxy-2,5,6-trichloroisophthalonitrile.

Critical end-points for setting guidance values for exposure to chlorothalonil and its metabolite SDS-3701 (4-hydroxy-2,5,6-trichloroisophthalonitrile)

	Chlorothalonil	SDS-3701
Absorption, distribution, excretion and metabolism in mammals		
Rate and extent of absorption	Rapid, incomplete and dose-dependent oral absorption (31% at 1.5–50 mg/kg bw; 13% at 200 mg/kg bw)	Rapid, incomplete oral absorption (26–30% at 4–62 mg/kg bw)
Distribution	Highest concentration in kidney (rat)	Percentage of administered dose in blood (5–6.9%), muscle (4.7–7.9%), fat (3.1–3.6%), liver (1–2%) and kidneys (0.4–0.7%) 4 days after dosing. Highest concentrations of radiolabel were found in liver.
Potential for accumulation	Low (rat)	Moderate, in view of amount in tissue after 4 days (rat)
Rate and extent of excretion	Plasma half-lives, 6–7 h at 5–50 mg/kg bw, >10 h at 200 mg/kg bw (rat)	75–82% in 4 days (rat)
Metabolism in animals	Extensive, metabolized by enzymatic processing of the diglutathione and triglutathione substituents via the mercapturic acid and cysteine conjugate β-lyase pathways, yielding *N*-acetyl cysteine, cysteinyl-glycine and *S*-methyl derivatives	No data
Toxicologically significant compounds (in animals, plants and the environment)	Chlorothalonil	SDS-3701
Acute toxicity		
LD_{50}, oral, rat	>5000 mg/kg bw	242–422 mg/kg bw
LD_{50}, dermal, rat	>5000 mg/kg bw	No data
LC_{50}, inhalation, rat	0.1 mg/l air	No data
Rat, dermal irritation	Not an irritant	No data
Rabbit, ocular irritation	Severely irritating	No data
Dermal sensitization	Inconclusive	No data
Short-term studies of toxicity		
Target/critical effect	Kidney (rat, rabbit)	Haematopoietic system (rat); body weight (dog)
Lowest relevant oral NOAEL	1.8 mg/kg bw per day (rat)	0.83 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	Systemic: 2.5 mg/kg bw per day (rabbit) Local: 2.5 mg/kg bw per day (rabbit)	No data
Long-term studies of toxicity and carcinogenicity		
Target/critical effect	Kidney: tubular epithelial necrosis/ hyperplasia (mouse, rat, dog)	Haematopoietic system (rat)
Lowest relevant NOAEL	1.8 mg/kg bw per day (rat)	3 mg/kg bw per day (rat)
Carcinogenicity	Carcinogenic, secondary to renal toxicity (mice, rats)	Not carcinogenic (mice, rats)
Genotoxicity		
	Not genotoxic	Not genotoxic

Reproductive toxicity		
Reproduction target/critical effect	No reproductive effects (rats)	No reproductive effects (rats)
Lowest relevant reproductive NOAEL	3000 ppm, equal to 138 mg/kg bw per day, highest dose tested (rats)	120 ppm, equivalent to 8 mg/kg bw per day, highest dose tested (rats)
Developmental target	Increased postimplantation loss, observed at maternally toxic doses only (rats)	Increased early and late postimplantation loss, decreased fetal weight, increased frequency of 14th rudimentary rib, observed at maternally toxic doses only (rats)
		Increased early postimplantation loss, observed at maternally toxic doses only (rabbits)
Lowest relevant developmental NOAEL	100 mg/kg bw per day (rats)	5 mg/kg bw per day (rats)
	20 mg/kg bw per day, highest dose tested (rabbits)	2.5 mg/kg bw per day (rabbits)
Neurotoxicity/delayed neurotoxicity		
Neurotoxicity	No data. No indication of neurotoxic potential	No data. No indication of neurotoxic potential
Medical data		
	Dermatitis reported in published literature	No data

Summary for chlorothalonil

	Value	Study	Safety factor
ADI	0–0.02 mg/kg bw	2-year study in rat	100
ARfD	0.6 mg/kg bw	Studies of acute toxicity, rat	100

Summary for SDS-3701

	Value	Study	Safety factor
ADI	0–0.008 mg/kg bw	1-year study, dog	100
ARfD	0.03 mg/kg bw	Study of developmental toxicity, rabbit	100

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Appendix 1: Application of the IPCS conceptual framework for cancer risk assessment

In chronic toxicity studies in mice and rats, kidney tumours, predominantly tubular adenomas and carcinomas, were observed. In rats, kidney tumours were observed at dietary doses equal to 15 mg/kg bw per day in males or at higher doses in both sexes. The overall NOAEL for kidney tumours in rats was 3.8 mg/kg bw per day. In male mice, kidney tumours were observed at doses of 119 mg/kg bw per day and higher. In a 2-year dog study (Paynter & Busey, 1966, evaluated by JMPR in 1992), renal glomerulosclerosis and degenerative renal tubular changes (tubular hypertrophy and dilatation) were observed at doses of 15 000 ppm, equivalent to 375 mg/kg bw per day, and higher. No kidney effects in dogs were observed at 1500 ppm, equivalent to 37.5 mg/kg bw per day.

Below, the mode of action for renal tumour formation is discussed using the structure of the IPCS conceptual framework.

A1. Postulated mode of action

Chlorothalonil is metabolized via initial glutathione conjugation and subsequent enzymatic processing of the diglutathione and triglutathione substituents to mercapturic acid and cysteine conjugates. The cysteine *S*-conjugates of chlorothalonil can be (actively) taken up, in particular in the S2 segment in renal tubular epithelial cells, and further processed via the β-lyase pathway, yielding reactive intermediates (thiols) causing cytotoxicity. Sustained damage due to prolonged exposure to chlorothalonil may result in regenerative renal cell hyperplasia and ultimately tumour formation.

A2. Key events

The key events in the chlorothalonil mode of action for kidney tumour formation in rodents include the following:

a) *The metabolism of chlorothalonil to glutathione conjugates, which are further processed to cysteine and mercapturic acid conjugates.* Evidence for this proposed metabolic pathway comes from the identification of the terminal *N*-acetyl cysteine, cysteinyl-glycine and *S*-methyl derivatives in urine of rats (see studies evaluated in the present monograph). There are several sites in the mammalian organism where the proposed metabolic conversions can occur, so it is not possible to determine where the different steps in the metabolic pathway occur (Wilkinson & Killeen, 1996).

b) *Uptake of cysteine S-conjugates of chlorothalonil in the epithelial cells of the convoluted proximal tubules in the kidneys*. Indirect evidence for such a process is provided in the database evaluated in the present monograph. Following oral administration of radiolabelled chlorothalonil, highest concentrations of radiolabel are found in the kidney. Moreover, it was shown that significant binding of radiolabel to kidney protein occurs, whereas covalent binding to DNA in kidney cells was not detected. The observation that probenecid, an inhibitor of the organic anion transporter, reduces urinary excretion of radiolabel by 50% indicates that active transport processes are involved in the renal excretion of chlorathonil derivatives in the urine. Data from published literature indicate that nephrotoxicity induced by cysteine *S*-conjugates is inhibited by probenecid (Dekant et al., 2001, 2003).

c) *Enzymatic processing of the cysteine S-conjugates of chlorothalonil via the β-lyase pathways, yielding reactive intermediates (thiols)*. No direct evidence for the formation of reactive intermediates is available. Although studies performed by Savides et al. (1986) and Marciniszyn et al. (1985b) suggested that, following oral administration of radiolabelled chlorothalonil, approximately 30% of the radiolabel in urine represented dithiol- or trithiol-substituted chlorothalonil, subsequent studies by Cuff, Kenna & Withe (2001) demonstrated that the thiol metabolites were probably an artefact of the analytical methodology. There is information from the published literature that other chlorinated compounds, through glutathione-dependent biotransformation and the cysteine *S*-conjugation β-lyase pathway, may be metabolized to a variety of reactive intermediates. Quantitative differences in some of the metabolic steps between rodents and other species, including humans, have been demonstrated. For instance, for some other compounds sharing this mode of action, kidney β-lyase activity is higher in rats than in other species (Lash et al., 1990; Iyer & Anders, 1996). However, specific information on chlorothalonil is not available.

d) *Renal cytotoxicity followed by regenerative cell proliferation*. Evidence for the occurrence of this process is provided by the studies evaluated in the present monograph. One study in rats showed that even 4 h after a single gavage dose of chlorothalonil at 175 mg/kg bw, there was electron microscopic evidence of focal epithelial swelling and stippled cytoplasmic vacuoles in the proximal convoluted tubules of the kidneys, and these effects were more severe 16 h after dosing. Light microscopic examination revealed tubular epithelial vacuolation 16 h after such a dose, and the incidence and severity of these findings were higher in animals treated for 2 days with chlorothalonil. In a second study with daily gavage treatment of rats with chlorothalonil for 4 days, a time-dependent effect on the S2 portion of the proximal convoluted tubules of the kidney was observed. After 1 day of dosing, single hypereosinophilic cells were observed in the S2 region of the proximal convoluted tubules. This progressed into a hydropic change affecting the whole S2 tubulus after 2, 3 or 4 days. The severity of the finding of eosinophilic cells gradually decreased with repeated dosing, whereas the severity of hydropic change increased. In addition, increased numbers of mitotic cells were present in the S2 proximal tubules after 2–4 days of

treatment. Chlorothalonil given in the diet at 175 mg/kg bw per day for up to 28 days induced vacuolation of the epithelium of the proximal tubules and increased PCNA staining at days 7, 14, 21 and 28. In a 3-month dietary study in rats, the effect of chlorothalonil treatment (175 mg/kg bw per day) on kidney cell proliferation at day 7, 28 or 91 was investigated using BrdU labelling. Cell proliferation in the kidneys was significantly higher in treated rats than in controls at all time points. The extent of the cell proliferation was highest at day 7 and decreased with time over the next two time points. In a second 3-month study in rats, administration of chlorothalonil caused kidney toxicity, which was evident as vacuolar degeneration of the proximal tubule seen after 4 days of dosing with progression to foci of basophilic tubules and/or proliferative interstitial fibrosis seen from 2 weeks onwards.

e) *Sustained damage due to prolonged exposure to chlorothalonil results in renal hyperplasia and ultimately tumour formation.* Evidence for the occurrence of this process is provided by the studies evaluated in the present monograph. In rat studies in which chlorothalonil was administered for 3 months or longer, renal tubular hyperplasia and hypertrophy were observed. In two chronic studies in rats, renal tumours were found. The incidence and severity of hyperplasia and tumour formation increased with dose and duration of treatment.

A3. Concordance of dose–response relationships

The observed cytotoxicity, regenerative cell proliferation, renal hyperplasia and kidney tumour formation occurred in a dose-related manner. Renal cytotoxicity occurs at lower doses than those causing tumours, and regenerative cell proliferation and kidney hyperplasia occurred at doses similar to those causing tumours.

A4. Temporal relationships

Based on studies of different durations and as described under key events d) and e), the temporal relationship of the observed events followed a logical pattern. The data indicate a sequential process starting with cytotoxicity, followed by cell proliferation and regenerative hyperplasia, ultimately resulting in kidney tumours.

A5. Biological plausibility and coherence

Cytotoxicity/regenerative proliferation is a well-established mode of action for the formation of kidney tumours. Based on information on other chlorinated hydrocarbons, it is possible that the nephrotoxicity caused by chlorothalonil may be due to reactive metabolites formed from the renal β-lyase cleavage of cysteine *S*-conjugates transported into the renal tubular cells. This mode of action is supported by the finding that when a monoglutathione conjugate of chlorothalonil is administered orally, similar kidney lesions are observed at a comparable dose.

A6. Other modes of action

Cytotoxicity/regenerative proliferation is a well-established mode of action for the formation of kidney tumours, although the cause of the initial cytotoxicity may differ. The metabolic profile of chlorothalonil, based on the identification of *N*-acetyl cysteine, cysteinyl-glycine and *S*-methyl derivatives in urine, is consistent with the hypothesis that nephrotoxicity caused by chlorothalonil may be due to reactive metabolites formed from the renal β-lyase cleavage of cysteine *S*-conjugates in the

renal tubular cells. Such a mode of action for renal tumour induction has been described for other chlorinated hydrocarbons.

An extensive range of in vitro and in vivo genotoxicity studies indicates that chlorothalonil is unlikely to be genotoxic. Thus, it can be concluded that the chlorothalonil-related renal tumours are induced through a non-genotoxic mode of action.

The possibility that the kidney tumours induced by chlorothalonil are a result of an α-2u-globulin-associated nephropathy mode of action can be ruled out, as, for instance, tumours were observed in both sexes, hyaline droplets in the kidneys were not reported and kidney tumours were observed in mice. Furthermore, there is no evidence to indicate that the chlorothalonil-induced nephrotoxicity and tumours are related to chronic progressive nephropathy.

A7. Human relevance

There is sufficient evidence to establish that the rodent renal tumours are due to a cytotoxic/regenerative proliferation mode of action that is dose and duration dependent. Further, the available data are suggestive of kidney tumour formation as a consequence of nephrotoxicity caused by reactive chlorothalonil metabolites formed from the renal β-lyase cleavage of cysteine *S*-conjugates in the renal tubular cells. The contribution of the β-lyase pathway to the renal effects of chlorothalonil has not been established unequivocally. All of the postulated key events for renal tumours are possible in humans. Hence, it was not possible to dismiss human relevance on the basis of qualitative differences between rodents and humans.

Because β-lyase activity is lower in human kidney tissue than in that of rodents, rodents would be expected to be more sensitive to this bioactivation pathway. A particular sensitivity of rats is also suggested by the observation that, in a 2-year dietary study in dogs, evaluated by JMPR in 1992, renal glomerulosclerosis and degenerative renal tubular changes (tubular hypertrophy and dilatation) were found only at high doses (≥375 mg/kg bw per day). The kidney toxicity in dogs following high doses of chlorothalonil only may also be due to species differences in absorption as well as bioactivation. However, there are insufficient data on chlorothalonil to specifically characterize quantitatively the differences in renal enzyme activity/bioactivation among rodents, dogs and humans. Thus, although rodents may be more susceptible than humans, the nephrotoxicity of chlorothalonil cannot be discounted in humans.

CHLORPYRIFOS-METHYL

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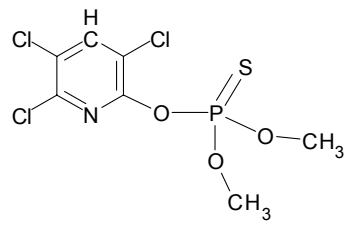
Explanation

Chlorpyrifos-methyl (Figure 1) is the International Organization for Standardization (ISO)–approved name for *O*,*O*-dimethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate (Chemical Abstracts Service [CAS] No. 5598-13-0). Chlorpyrifos-methyl is an organophosphorus compound that acts

against insects. The mechanism of action is inhibition of acetylcholinesterase activity. Chlorpyrifos-methyl was evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1975, 1991 and 1992, when an acceptable daily intake (ADI) of 0–0.01 mg/kg body weight (bw) was established. In 2001, the Meeting concluded that an acute reference dose (ARfD) for chlorpyrifos-methyl was not necessary. Chlorpyrifos-methyl was reviewed at the present Meeting as part of the periodic review programme of the Codex Committee on Pesticide Residues. New studies of dermal and inhalation exposure in rats, genotoxicity in vivo, reproductive toxicity and inhibition of neuropathy target esterase (NTE) had been made available since the last full review in 1992.

Figure 1. Structure of chlorpyrifos-methyl



Most of the pivotal studies met the basic requirements of the relevant Organisation for Economic Co-operation and Development (OECD) or national test guidelines, although the level of detail in some of the reports did not always match current requirements. A number of studies did not contain certificates of compliance with good laboratory practice (GLP). The available studies in human volunteers were considered to have been performed according to contemporary ethical standards. The overall database is considered adequate for deriving reference doses.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

Rats

In a 1971 (non-GLP, non-guideline) study, two male Sprague-Dawley rats (~200 g) were gavaged with ^{14}C-radiolabelled chlorpyrifos-methyl (purity >99%; 377 MBq/mmol; labelled at positions 2 and 6 on the pyridyl ring) at a dose level of 16 mg/kg bw (in corn oil). Urine, faeces and expired carbon dioxide were collected for up to 72 h. Blood samples were taken from the tail at intervals throughout the study period. Radioactivity levels were determined by liquid scintillation counting or combustion analysis (faecal samples). Urinary metabolites were identified by thin-layer chromatography (TLC) and autoradiography.

Peak blood radioactivity levels were reported at 5 h, at which point the amount of test material in the blood was calculated to be approximately 3.0% (2.4–3.7%; absolute levels not given) of the administered dose. Levels of radioactivity indicated rapid clearance from blood, and the peak level seen at 5 h had declined by 50% at approximately 9 h following administration, suggesting an initial half-life of approximately 4 h. Excretion of radioactivity was found to be primarily in the urine (Table 1) and largely within 24 h of administration. Radioactivity levels in expired air were minimal.

Table 1. Excretion of radioactivity following a single oral dose of chlorpyrifos-methyl (16 mg/kg bw) to rats

Parameter	Radioactivity excreted in 0–72 h (% of administered dose)
Urine	83–85
Faeces	7–9
Expired air	0.23–0.43
Total residual	0.65–1.3
Total recovery	91–95

From Branson & Litchfield (1970)

Table 2. Residual tissue radioactivity at 72 h following a single oral dose of chlorpyrifos-methyl (16 mg/kg bw) to rats

Tissue	Radioactivity (mg equivalent/kg)	
	Animal A	Animal B
Red blood cells	0.013	0.017
Serum	nd	nd
Liver	0.075	0.108
Spleen	0.039	0.021
Kidneys	0.035	0.074
Adrenals	0.038	0.342
Heart	0.003	0.019
Brain	0.005	0.006
Muscle	0.043	0.164
Fat	0.057	0.156
Bone	0.041	0.634

From Branson & Litchfield (1970)
nd, not detected

Residual tissue radioactivity levels at 72 h were low in all tissues (<1 part per million [ppm]), but varied markedly between the two animals (Table 2) (Branson & Litchfield, 1970).

In a 1976 (non-GLP, non-guideline) study, 10 male rats (strain not specified; ~330 g) were gavaged with a single dose of approximately 30 mg/kg bw of [^{14}C]chlorpyrifos-methyl (radiopurity >99%; 25.5 kBq/rat) in 0.5 ml ethanol. Urine and faeces were collected for up to 48 h, when rats were terminated. Radioactivity was quantified by scintillation counting (urine) or combustion analysis (faeces). Overall recovery was low (<80%). Radioactivity was found to be excreted primarily in urine (mean 64% of the administered dose) and largely within 24 h of administration. No tissue residues were detected at 48 h (Bakke & Price, 1976).

A briefly reported study of the comparative kinetics of chlorpyrifos (Dowco 179) and chlorpyrifos-methyl (Dowco 214) in rats showed chlorpyrifos to be more rapidly absorbed (time to peak concentration in blood [T_{max}] = 3 h) than chlorpyrifos-methyl (T_{max} = 5 h). Both compounds had similar peak concentrations in blood (C_{max} values). Chlorpyrifos was excreted more rapidly than chlorpyrifos-methyl, but plasma levels of radiolabel from both compounds were very low at 24 h post-dosing (Branson & Litchfield, 1970).

Table 3. Recovery of radioactivity from rat skin samples (n = 6) exposed to [^{14}C]chlorpyrifos-methyl after 28 h

Sample	Mean recovery of radioactivity (%)	
	Concentrate	1% aqueous dilution
Receptor fluid	3.32 ± 0.29	1.08 ± 0.48
Skin	23.40 ± 6.46	32.55 ± 3.06
Surface wash	66.63 ± 8.31	60.12 ± 5.12
Cell wash	4.37 ± 1.32	4.11 ± 1.29
Additional vial wash	0.17 ± 0.15	0.26 ± 0.18
Total mass balance	97.9	98.1

From Perkins (1995)

(b) Dermal route

The dermal absorption of Reldan 22 (224 g/l chlorpyrifos-methyl, solvent based) was assessed in vitro using full-thickness male Fischer 344 rat skin. Single (10 µl) 28 h applications of ^{14}C-labelled test material (undiluted or 1% aqueous dilution) were made to the epidermal surface of skin discs held in occluded flow diffusion cells at 31 °C. Receptor fluid (tissue culture medium containing 6% polyethylene glycol 20 oleyl ether) was collected at 2 h intervals. The solubility of chlorpyrifos-methyl in the receptor fluid was confirmed to be higher than the applied dose. For the concentrate, total recovery of radioactivity was high (92.27–99.32%). A lag time for penetration of 4–6 h was observed; this was followed by linear absorption over the following 24 h. For the spray strength dilution, total recovery of radioactivity was also high (95.05–100.89%). Distribution of radioactivity was consistent for all of the six replicates. A lag time for penetration of 8–10 h was observed; this was followed by linear absorption over the remainder of the study. The linear absorption rate was calculated to be 10.4 µg/cm^2 per hour for the concentrate and 0.039 µg/cm^2 per hour for the 1% dilution. Dermal penetration was low for both formulation types (Table 3) (Perkins, 1995).

1.2 Biotransformation

Rats

The urinary metabolites of chlorpyrifos-methyl were investigated by TLC using urine samples collected as part of a previously described study (Branson & Litchfield, 1970). The primary metabolite was identified by co-chromatography as 3,5,6-trichloro-2-pyridinol (TCP). Additional non-mobile material was stated to be conjugated TCP. Metabolites were not quantified, and faecal metabolites were not investigated (Branson & Litchfield, 1970).

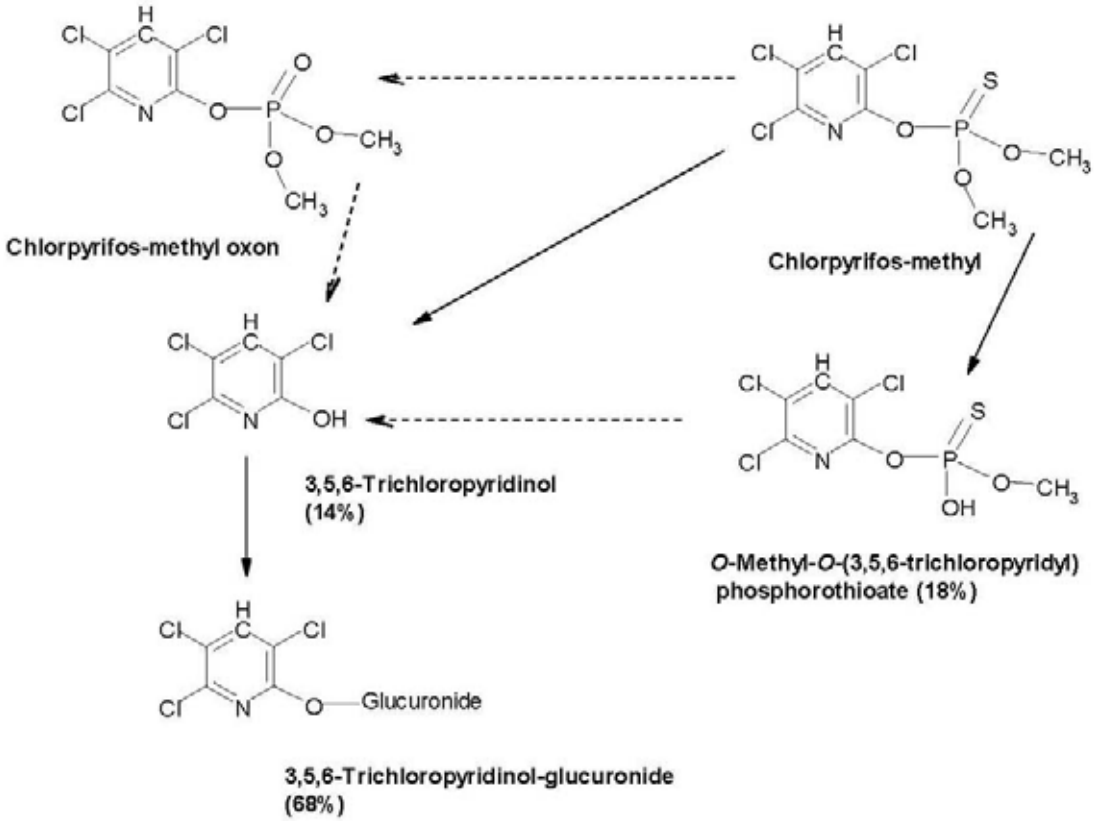
Urinary metabolites from the study of Bakke & Price (1976) were identified by gas–liquid chromatography, TLC and mass spectrometry as TCP (13.8%), its glucuronide conjugate (68.6%) and the desmethyl metabolite *O*-methyl-*O*-(3,5,6-trichloropyridyl) phosphorothioate (17.8%). Faeces were not analysed, as they contained less than 5% of the administered radiolabel (Bakke & Price, 1976).

The above results, although minimally reported, are broadly consistent with data for the closely related compound, chlorpyrifos (see Annex 1, reference *86*).

A normal metabolic reaction with phosphorothioates is oxidative desulfuration or isomerization to give the oxon form, which is the more potent inhibitor of acetylcholinesterase. Chlorpyrifos-methyl oxon was not identified in the rat metabolism studies with chlorpyrifos-methyl.

A proposed metabolic pathway is shown in Figure 2.

Figure 2. Proposed metabolic pathway of chlorpyrifos-methyl in rats (% of administered radiolabel in parentheses)



2. Toxicology studies

2.1 Acute toxicity

(a) Lethal doses

Chlorpyrifos-methyl was of low acute toxicity via the oral, dermal and inhalation routes (Table 4).

(b) Dermal and ocular irritation and dermal sensitization

Chlorpyrifos-methyl was a slight transient irritant to rabbit skin (Jones, 1984) and eyes (Jones, 1985c). In a guinea-pig skin sensitization study using a Buehler protocol, chlorpyrifos-methyl was not a skin sensitizer (Jones, 1985d), but positive results were seen when a Magnusson & Kligman maximization protocol was used (Wilson, 2000).

2.2 Short-term studies of toxicity

(a) Oral route

Mice

Chlorpyrifos-methyl (purity 91.8%; batch No. AGR 209075) was administered to ICR(Crj:CD-1) mice (12 of each sex per group) in the diet at a level of 0, 1, 5, 10, 1000 or 10 000 ppm for 28 days. Blood samples were taken at termination for the measurement of haematological and

Table 4. Acute toxicity studies with chlorpyrifos-methyl

Test substance	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Purity (%); batch	Vehicle	Reference
OP2	Rat	SD	M & F	Oral	2814	—	95.5; EK 8429097	Corn oil	Lackenby (1985)
Reldan F	Rat	SD	M & F	Oral	>5000	—	95.5	Polyethylene glycol	Jones (1985a)
Chlorpyrifos-methyl	Mouse	OF-1	M & F	Oral	2843	—	96.9	0.5% CMC	Clouzeau (1992)
Reldan F	Rat	SD	M & F	Dermal	>2000	—	95.5; EK 840929097	Polyethylene glycol	Jones (1985b)
Reldan F	Rat	HC/ CFHB	M & F	Inhalation (4 h, nose only)	—	>0.67[a]	Not stated; EK 82092 8086	None; vapour (55 °C)	Hardy & Jackson (1984)

CMC, carboxymethyl cellulose; F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male; SD, Sprague-Dawley
[a] Maximal technically achievable concentration.

clinical chemistry parameters (five of each sex per group) and assessment of cholinesterase activity (six of each sex per group). The method of determination of cholinesterase activity was an automated 5,5′-dithiobis(2-nitrobenzoic) acid (DTNB) procedure based on the method of Ellman et al. (1961). The brain, pituitary, thyroid, heart, thymus, liver, kidneys, spleen, adrenals and gonads were weighed at necropsy. A comprehensive list of tissues from animals of the control, 1000 and 10 000 ppm groups were subjected to histopathological examination.

Dietary analyses revealed satisfactory stability, homogeneity and concentration of test material. Achieved intakes were 0, 0.12, 0.65, 1.3, 122 and 523 mg/kg bw per day in males and 0, 0.14, 0.75, 1.5, 139 and 318 mg/kg bw per day in females. All animals administered 10 000 ppm were found dead or killed in extremis on days 5–9. The report authors cite starvation of these animals due to the severe unpalatability of test material in the diet. There were no deaths or clinical signs in the lower dose groups. Body weights of 1000 ppm animals of both sexes were slightly lower throughout the study period; values attained statistical significance for males at week 4. Overall food consumption, water consumption and food conversion efficiency were slightly (but not statistically significantly) decreased at 1000 ppm.

Urinalysis and haematology did not reveal any treatment-related findings. Clinical chemistry findings indicative of mild hepatotoxicity were noted at the top dose level in both sexes. Plasma cholinesterase activity was significantly lower at 10 ppm and above in males and at 5 ppm and above in females. Erythrocyte cholinesterase activity was significantly lower at 10 ppm and above in males and at 1000 ppm only in females. Brain cholinesterase activity was significantly reduced at 1000 ppm in both sexes (Table 5).

Mean absolute and relative liver and adrenal weights were significantly increased in 1000 ppm males (~25%); relative liver weight was also significantly increased in 1000 ppm females (~12%). Emaciation of all 10 000 ppm decedents was noted at necropsy, together with splenic atrophy, hepatocellular atrophy and pancreatic acinar cell atrophy. Adrenal cortical cell swelling in the zona fasciculata was noted in all 1000 ppm males; similar findings were not reported in females.

A no-observed-adverse-effect level (NOAEL) of 10 ppm, equal to 1.3 and 1.5 mg/kg bw per day in males and females, respectively, can be determined for this study, based on decreased brain cholinesterase activity and adrenal and liver pathology at 1000 ppm (Yoshida et al., 1985).

Table 5. Clinical chemistry parameters and cholinesterase activity in mice receiving chlorpyrifos-methyl in the diet for 28 days

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	1	5	10	1000	0	1	5	10	1000
GPT (U/l)	13	11	11	10	17	8	14	7	11	12*
Cholesterol (mg/dl)	93	104	108	100	149	82	70	84	86	127**
Plasma cholinesterase[a]										
U/ml	3.59	3.54	2.66	1.69*	0.15***	5.40	4.85	4.46*	4.14**	0.14***
% of control	—	99	74	47	4	—	90	83	77	3
Red blood cell cholinesterase[a]										
U/ml	0.15	0.16	0.15	0.10*	0.00***	0.13	0.13	0.12	0.11	0.00***
% of control	—	107	100	67	0	—	100	92	85	0
Brain cholinesterase[a]										
U/ml	0.28	0.25	0.24	0.25	0.08***	0.23	0.22	0.24	0.24	0.06***
% of control	—	89	86	89	29	—	96	104	104	27

From Yoshida et al. (1985)
GPT, glutamic–pyruvic transaminase; U, unit; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$
[a] Activity at termination.

Rats

In a 1975 (non-GLP) study, Sprague-Dawley rats (five of each sex per dose level) were gavaged with Dowco 214 (purity and batch not specified), suspended in 1% gum tragacanth, at 0, 0.2, 1 or 5 mg/kg bw per day, 6 days/week, for 6 weeks. Blood was sampled from the orbital sinus after 30 days of dosing for the determination of cholinesterase activity according to the pH stat method of Nabb & Whitfield (1967). Aortal blood samples were taken at termination for the assessment of haematological and clinical chemistry parameters. Liver samples were taken for the determination of mixed-function oxidase (*N*-demethylase and biphenyl hydroxylase) activity. Homogenized whole-brain samples were assayed for acetylcholinesterase activity.

Overall body weight gain in top-dose females was slightly reduced (81% of controls). No effects were seen in other groups. No effects were seen on haematological or clinical chemistry parameters at any dose level. No consistent effects were seen on mixed-function oxidase activity. Plasma and erythrocyte cholinesterase activities were reduced at 1 mg/kg bw per day and higher. A trend to decreasing brain cholinesterase activity was noted in all dose groups, with reductions of greater than 20% at 5 mg/kg bw per day (Table 6). None of the decreases in cholinesterase activity were reported to be statistically significant.

A NOAEL of 1 mg/kg bw per day can be determined for this study, based on decreased (>20%) brain cholinesterase activity at 5 mg/kg bw per day, although this was not statistically significant. The study report does not identify the relative times of cholinesterase measurement and the final dose (Griffin & Coulston, 1975).

Table 6. Cholinesterase activity in a 6-week study in rats administered chlorpyrifos-methyl in the diet

Cholinesterase sample	Time	Cholinesterase activity (μmol/min per milligram protein, mean ± SD)							
		Dose (mg/kg bw per day)							
		0		0.2		1		5	
		M	F	M	F	M	F	M	F
Plasma	Pretest	0.9 ± 0.1	1.8 ± 0.9	0.6 ± 0.1	1.7 ± 0.6	0.5 ± 0.0	1.8 ± 0.4	0.7 ± 0.1	1.4 ± 0.2
	Day 30	0.5 ± 0.1	2.4 ± 1.5	0.5 ± 0.1	2.1 ± 0.7	0.4 ± 0.1	1.4 ± 0.4	0.4 ± 0.1	0.9 ± 0.2
	(%)[a]	—	—	100	88	80	58	80	68
Red blood cells	Pretest	1.1 ± 0.1	1.6 ± 0.2	1.8 ± 0.8	2.5 ± 0.2	1.9 ± 0.3	2.2 ± 0.7	1.7 ± 0.7	2.6 ± 1.0
	Day 30	1.9 ± 0.2	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.2	1.5 ± 0.3	2.0 ± 0.1	1.1 ± 0.2	1.8 ± 0.5
	(%)[a]	—	—	111	100	79	95	58	86
Brain	Termination	0.092 ± 0.013	0.114 ± 0.010	0.081 ± 0.011	0.108 ± 0.006	0.083 ± 0.017	0.102 ± 0.013	0.070 ± 0.010	0.091 ± 0.010
	(%)[a]	—	—	88.0	94.7	90.2	89.5	76.1	79.8

From Griffin & Coulston (1975)
F, female; M, male; SD, standard deviation
[a] % of day 30 control.

Fischer 344 rats (10 of each sex per group) were administered Reldan (purity ~95%; batch No. AGR 219561) in the diet at variable dose levels calculated to be equivalent to 0, 0.1, 1, 10 or 250 mg/kg bw per day for 13 weeks. Additional recovery groups (10 of each sex) at 0 and 250 mg/kg bw per day were similarly treated, then administered control diet for an additional 4 weeks. Blood samples for the analysis of haematological and clinical chemistry parameters were taken from the orbital sinus immediately prior to termination. Samples for urinalysis were also taken prior to termination. Plasma and erythrocyte cholinesterase activities were measured in blood samples taken during week 6 and at termination. Brain cholinesterase activity was determined in half-brain samples taken at necropsy. Cholinesterase activity was measured using an automated procedure based on the method of Ellman et al. (1961). A functional observational battery (FOB) was performed on days 10, 30, 86 and 115. The adrenals, brain, heart, kidneys, liver and gonads were weighed at necropsy. The adrenals, brain, spinal cord, peripheral nerve, liver, kidney, lungs and gross lesions from all animals were examined microscopically. A comprehensive list of tissues from the control and top dose group were similarly examined.

Dietary analyses revealed acceptable homogeneity, stability and concentration of test material. No animals died during the study period. Food consumption was consistently lower in both sexes at the top dose level; however, values did not attain statistical significance. Food consumption during the recovery period was comparable in both groups. Body weights and weight gains of top-dose animals of both sexes were significantly lower than those of controls throughout the study period (Table 7). No consistent effects were seen at lower dose levels. Body weights of top-dose females remained significantly lower than those of controls after the recovery period; however, body weight gain was increased in both sexes.

Plasma cholinesterase activity was significantly decreased at 1 mg/kg bw per day and higher in both sexes at weeks 6 and 13 and in 0.1 mg/kg bw per day males at week 13 only. Week 13 values for treated groups of males were slightly but consistently lower than week 6 values; similar effects were not seen in females. Following the recovery period, activities showed evidence of considerable recovery. Erythrocyte cholinesterase activity was significantly decreased in both sexes at 10 mg/kg bw per day and higher at week 6 and in 250 mg/kg bw per day females only at week 13. Activities at weeks 6 and 13 were comparable in both sexes. Activities at week 13 in males at 10 mg/kg bw per day and

Table 7. Body weights and body weight gains in rats exposed to chlorpyrifos-methyl in the diet for 13 weeks

Parameter	Day	Body weight or body weight gain (g)									
		Males					Females				
		Dose (mg/kg bw per day)									
		0	0.1	1	10	250	0	0.1	1	10	250
Body weight	14	194.7	194.7	193.8	189.2	186.2*	132.0	129.7	131.1	132.3	125.0*
	42	266.1	263.5	260.5	257.4	246.3*	169.7	167.2	168.7	166.3	151.0*
	91	321.0	312.2	313.1	308.3	289.4*	193.0	190.6	190.7	186.8	166.3*
Body weight gain	0–91	194.5	185.5	187.1	183.5	161.1*	95.4	94.5	94.0	89.5	70.3*
Body weight	112	327.0				315.2	193.7				179.9*

From Barna-Lloyd, Szabo & Davis (1990)
* P □ 0.05

higher and in females at 10 mg/kg bw per day were reduced, but did not attain statistical significance. Some evidence of recovery in erythrocyte acetylcholinesterase activity was seen at week 17, although values for treated animals remained significantly lower than those of controls. Brain cholinesterase activity was significantly decreased in both sexes at 10 mg/kg bw per day and higher (Table 8). Evidence of significant recovery was seen 4 weeks after cessation of treatment.

Red blood cell count, haemoglobin content and packed cell volume were significantly decreased in both sexes at 250 mg/kg bw per day. Evidence of recovery was seen; however, some parameters remained significantly lower than controls. Platelet count was significantly higher in both sexes at 250 mg/kg bw per day; changes in females at lower doses lacked a clear dose–response relationship and are not considered to be of toxicological significance. Some evidence of recovery was seen at week 17. Significantly lower alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (AP) activities at the top dose level are not considered to be of toxicological significance in their own right and are possibly secondary to reduced body weight gain. No notable changes were evident at 10 mg/kg bw per day. Urinalysis did not reveal any treatment-related findings.

For the FOB investigations, decreased condition of hair, increased urine staining and lacrimation were observed at the top dose level. Tremor was noted in one top-dose female at the day 30 observation. No behavioural effects were noted in any animals. Mean absolute and relative adrenal, kidney and liver weights were significantly increased in both sexes at the top dose level. Mean relative adrenal weights were also increased at 10 mg/kg bw per day in both sexes. With the exception of kidney weights in males, organ weights in treated animals were considerably lower following the recovery period.

No treatment-related macroscopic findings were noted at necropsy. Microscopically, bilateral diffuse hypertrophy and vacuolation of the adrenal cortex zona fasciculata were noted in all animals at 10 and 250 mg/kg bw per day (Table 9). The severity of these findings was decreased following the recovery period. Adrenal findings were stated by the report authors to be consistent with lipid accumulation. Adrenal necrosis with accompanying inflammation was also noted in females at week 13 only. Degeneration/regeneration of the renal tubules was noted with increased severity in males at 250 mg/kg bw per day. Very slight hepatic centrilobular hypertrophy was also noted in this group. Microscopic renal and hepatic findings were not apparent following the recovery period.

Administration of Reldan to rats for 90 days resulted in marked inhibition of cholinesterase activities. Evidence of the reversibility of signs of toxicity, cholinesterase inhibition, organ weight

Table 8. Cholinesterase activity in rats exposed to chlorpyrifos-methyl in the diet for 13 weeks

Cholinesterase sample	Week	Cholinesterase activity									
		Males					Females				
		Dose (mg/kg bw per day)									
		0	0.1	1	10	250	0	0.1	1	10	250
Plasma, U/ml	6	0.649	0.649	0.568*	0.478*	0.333*	2.82	2.93	1.86*	0.803*	0.359*
(% of control)		(—)	(100)	(88)	(74)	(51)	(—)	(104)	(66)	(28)	(13)
	13	0.631	0.585*	0.502*	0.418*	0.292*	2.66	3.01	1.76*	0.721*	0.358*
		(—)	(93)	(80)	(66)	(46)	(—)	(113)	(66)	(27)	(13)
	17	0.590				0.549*	3.193				3.132
		(—)				(93)	(—)				(98)
Red blood cells,	6	2.18	2.04	1.84	1.62*	1.52*	2.43	2.24	2.14	1.66*	1.81*
U/ml		(—)	(94)	(84)	(74)	(70)	(—)	(92)	(88)	(68)	(74)
(% of control)	13	2.25	2.52	2.07	1.91	1.87	1.52	1.50	1.58	1.36	1.10*
		(—)	(112)	(92)	(85)	(83)	(—)	(99)	(104)	(89)	(72)
	17	2.18				1.92*	2.04				1.68*
		(—)				(88)	(—)				(82)
Brain, U/g	13	10.55	10.31	10.15	8.77*	3.95*	10.35	10.32	10.19	8.84*	4.26*
(% of control)		(—)	(98)	(96)	(83)	(37)	(—)	(100)	(98)	(85)	(41)
	17	10.38				8.38*	10.55				8.73*
		(—)				(81)	(—)				(83)

From Barna-Lloyd, Szabo & Davis (1990)
* $P \leq 0.05$

effects and microscopic findings was seen after a 4-week recovery period. A NOAEL of 1 mg/kg bw per day can be determined for this study, based on decreased brain cholinesterase activity and adrenal microscopic findings at 10 mg/kg bw per day and higher (Barna-Lloyd, Szabo & Davis, 1990).

Dogs

Beagle dogs (four of each sex per group) were administered Reldan (purity 95.2%) in the diet at dose levels calculated to be equivalent to 0, 0.1, 10 or 50 mg/kg bw per day for 13 weeks. Blood samples were taken prior to the study and on days 36 and 85 for the assessment of haematological and clinical chemistry parameters and blood and plasma cholinesterase activities. Urinalysis was performed at necropsy. The adrenals, brain, kidneys, liver, pituitary, gonads and thyroid were weighed at necropsy. A comprehensive list of tissues from all animals were subjected to histopathology. Brain cholinesterase activity was measured at necropsy. Cholinesterase activities were measured using an automated procedure based on the method of Ellman et al. (1961).

Dietary analyses revealed that concentration, stability and homogeneity of the test material were within acceptable limits. No animals died during the study period. Weakness and severe muscle wasting were noted in one top-dose female; this animal was killed on day 85. Moderate muscle wasting was noted in an additional animal in this group. Decreased weight gain was noted in top-dose males. Mean body weights of top-dose males were lower from day 28; one animal lost weight over the study period, and intermittent weight loss was noted in another animal. Mean body weights of top-dose females were lower than those of controls from day 14. Weight gain over the study period was noted in only one animal in this group; other animals lost weight over the study period. No effects on body weight or body weight gain were noted at lower dose levels. Food consumption at 50 mg/kg bw per day was lower in both sexes (15–30%).

Table 9. Microscopic findings in rats (n = 10) exposed to chlorpyrifos-methyl in the diet for 13 weeks

Finding	Severity	Week	Incidence									
			Males					Females				
			Dose (mg/kg bw per day)									
			0	0.1	1	10	250	0	0.1	1	10	250
Adrenal hypertrophy	Slight	13	0	0	0	10	0	0	0	0	9	0
		17	0				10	0				0
	Moderate	13	0	0	0	0	10	0	0	0	1	10
		17	0				0	0				0
Adrenal vacuolation	Very slight	13	0	0	0	0	0	0	0	0	0	0
		17	0				0	0				10
	Slight	13	0	0	0	0	0	0	0	0	10	0
		17	0				10	0				0
	Moderate	13	0	0	0	0	10	0	0	0	0	10
		17	0				0	0				0
Adrenal necrosis		13	0	0	0	0	0	0	0	0	2	8
Kidney tubule degeneration	Very slight	13	10	10	10	9	1	1	1	0	1	0
	Slight	13	0	0	0	0	9	0	0	0	0	0
Centrilobular hypertrophy	Very slight	13	0	0	0	0	7	0	0	0	0	0

From Barna-Lloyd, Szabo & Davis (1990)

Red blood cell count, packed cell volume and haemoglobin concentration were significantly decreased in both sexes at the top dose level at termination. Platelet numbers were significantly increased in both sexes at this dose level. AP, ASAT and creatine kinase activities were increased at the top dose level. Significant decreases in blood urea nitrogen, plasma creatinine, glucose, total protein, albumin, globulin, cholesterol and triglyceride levels seen in this group are considered to be secondary to weight loss and decreased food consumption in these animals. There were no notable effects at 10 mg/kg bw per day or below.

A dose-related decrease in plasma cholinesterase activity was seen at all dose levels in both sexes. Erythrocyte cholinesterase activities were reduced at all dose levels in both sexes; however, effects at 0.1 mg/kg bw per day were marginal. Plasma and erythrocyte cholinesterase activities at weeks 6 and 13 were comparable. Brain cholinesterase activity was significantly reduced at 50 mg/kg bw per day only (Table 10).

Mean relative liver and kidney weights were significantly increased in both sexes at the top dose level. Effects on kidney weight may reflect the lower body weights of animals in these groups. Gross findings at necropsy were limited to decreased mesenteric fat and skeletal muscle atrophy in two top-dose females. Microscopically, slight vacuolation of the renal proximal tubules was noted in top-dose females. Slight, diffuse centrilobular hepatocyte hypertrophy was noted in both sexes at the top dose level, skeletal muscle degeneration was noted in females at the top dose and thymic atrophy was observed in all top-dose males. No changes in histopathology of the adrenals were noted. No treatment-related findings were noted at lower dose levels.

A NOAEL of 10 mg/kg bw per day can be determined for this study, based on clinical signs, body weight effects, haematological and clinical chemistry findings, histopathological effects and

Table 10. Cholinesterase activities in dogs exposed to chlorpyrifos-methyl in the diet for 13 weeks

Cholinesterase sample	Time	Cholinesterase activity							
		Males				Females			
		Dose (mg/kg bw per day)							
		0	0.1	10	50	0	0.1	10	50
Plasma, U/ml (% of control)	Week −1	2.244	2.313	2.298	2.300	2.424	2.409	2.260	2.253
	Week 6	1.984	1.764	0.967	0.460	2.169	1.873	0.844	0.501
		(—)	(89)	(49)	(23)	(—)	(86)	(39)	(23)
	Week 13	1.781	1.773	0.798	0.427	1.924	1.653	0.822	0.478
		(—)	(100)	(45)	(24)	(—)	(86)	(43)	(25)
Red blood cells, U/ml (% of control)	Week −1	3.035	3.025	2.725	2.900	3.000	2.675	2.935	2.875
	Week 6	2.360	2.235	1.690	1.630	2.440	2.415	1.725	1.640
		(—)	(95)	(72)	(69)	(—)	(99)	(71)	(67)
	Week 13	1.805	2.040	1.350	1.485	1.890	1.775	1.520	1.395
		(—)	(113)	(75)	(82)	(—)	(94)	(70)	(74)
Brain, U/g (% of control)	Week 13	4.40	4.81	4.48	1.48**	4.95	4.77	4.39	1.48**
		(—)	(109)	(102)	(34)	(—)	(96)	(89)	(30)

From Szabo & Davis (1990)
U, units; ** □ = 0.05 (analysis of variance)

inhibition of brain cholinesterase activity at 50 mg/kg bw per day. Animals were fasted overnight prior to termination; it is therefore possible that cholinesterase inhibition was underestimated in this study (Szabo & Davis, 1990).

Monkeys

In a 1975 (non-GLP) study, Rhesus monkeys (three of each sex per group) were gavaged with Dowco 214 (batch and purity not specified), suspended in gum tragacanth, at 0, 0.1, 0.2, 1 or 5 mg/kg bw per day, 6 days/week, for 26 weeks. Weekly blood samples were taken for the assessment of plasma and erythrocyte cholinesterase activities from 6 weeks pre-exposure according to the method of Nabb & Whitfield (1967). Samples were also taken for the assessment of routine haematological and clinical chemistry parameters at intervals throughout the study. Liver samples were taken at necropsy for the determination of mixed-function oxidase (*N*-demethylase and biphenyl hydroxylase) activity. Samples of mid-brain were taken for the determination of cholinesterase activity. The heart, liver, brain and kidneys were weighed at necropsy, and a comprehensive list of tissues were evaluated microscopically, including sciatic nerve, cervical spinal cord, medulla, pons, optic chiasm, cerebellum and cerebrum using haematoxylin and eosin (H&E) staining.

One male at 1 mg/kg bw per day was terminated early during the study owing to a persistent infection. There were no further deaths. No clinical signs of toxicity were reported during the study period, and no effects were seen on body weight. No treatment-related effects were seen on haematological or clinical chemistry parameters. No effects were seen on mixed-function oxidase activity. Plasma cholinesterase activities showed considerable intraindividual and interindividual variation, but were decreased in all groups throughout the study period. Consistent decreases in activity were seen at 2 mg/kg bw per day and higher in males and at 1 mg/kg bw per day and higher in females. Erythrocyte cholinesterase activities were less variable; decreased activity (~60% inhibition) was seen in both sexes at the top dose level (Table 11). No consistent time-related change was seen in plasma or erythrocyte cholinesterase activity. Brain cholinesterase activity was not consistently

Table 11. Cholinesterase activity in monkeys administered chlorpyrifos-methyl for 26 weeks

Cholinesterase sample	Time	Cholinesterase activity, μmol/min per milligram protein (% of control)									
		Dose (mg/kg bw per day)									
		0		0.1		1		2		5	
		M	F	M	F	M	F	M	F	M	F
Plasma	Pretest[a]	11.40	11.50	7.57	12.53	10.30	7.80	6.80	9.13	7.17	11.00
	Week 6	9.07	8.90	6.57	9.67	8.27	6.30	4.10	4.73	2.17	3.00
		(—)	(—)	(72)	(109)	(91)	(71)	(45)	(53)	(24)	(34)
	Week 13	10.40	9.50	8.47	11.60	10.23	6.63	5.30	5.77	3.40	3.10
		(—)	(—)	(81)	(122)	(98)	(70)	(51)	(61)	(33)	(33)
	Week 27	8.97	8.93	6.90	10.47	8.70	5.63	3.95	5.20	2.53	2.50
		(—)	(—)	(77)	(117)	(97)	(63)	(76)	(58)	(28)	(28)
Red blood cells	Pretest[a]	11.57	12.60	12.73	12.17	12.57	12.80	11.53	12.97	10.33	12.43
	Week 6	11.67	12.40	12.83	12.13	12.80	12.83	10.63	11.60	3.17	4.17
		(—)	(—)	(110)	(98)	(110)	(103)	(91)	(94)	(27)	(34)
	Week 13	11.50	12.87	13.13	11.90	11.70	12.43	12.33	11.17	2.57	3.20
		(—)	(—)	(114)	(92)	(102)	(97)	(107)	(87)	(22)	(25)
	Week 27	12.83	11.37	12.40	11.40	11.50	11.23	9.50	11.37	3.20	3.00
		(—)	(—)	(97)	(100)	(90)	(99)	(74)	(100)	(25)	(26)
Brain	Week 27	0.106	0.099	0.106	0.114	0.119	0.101	0.115	0.107	0.090	0.110
		(—)	(—)	(100)	(108)	(112)	(95)	(109)	(101)	(85)	(104)

From Griffin & Coulston (1975)

[a] Mean of six pretest time points.

affected at any dose level. No effects on organ weights were noted at necropsy. No treatment-related gross or microscopic findings were noted.

A NOAEL of 5 mg/kg bw per day can be determined for this study in the absence of any treatment-related adverse effects at the top dose level (Griffin & Coulston, 1975).

(b) Dermal route

Rats

Groups of Fischer 344 rats (10 of each sex) were exposed dermally to Reldan F (purity 96.8%; lot No. NB05272036) at levels of 0, 10, 100 or 300 mg/kg bw per day, 6 h/day, 7 days/week, for 28 days, to evaluate the potential for systemic toxicity. Reldan F was applied in 0.5% methyl cellulose and covered with a semiocclusive dressing. An additional 10 male and 10 female rats in the control and high dose groups were untreated for an additional 2 weeks following the treatment period to assess recovery. Regular observations (including dermal), ophthalmology examinations, detailed clinical observations, body weight, food consumption, haematology (including prothrombin time), clinical chemistry (including cholinesterase activity), urinalysis and organ weights were evaluated. In addition, a gross necropsy was conducted, with extensive histopathological examination of tissues.

There were no deaths during the study. Cage-side observations of the dosing-phase animals revealed no abnormal findings. The only treatment-related alteration was periocular soiling at days 22 and 28 in four females given 300 mg/kg bw per day. Local irritant effects were seen from day 8 onwards at 100 and 300 mg/kg bw per day; histopathology identified the responses as hyperplasia, hyperkeratosis and inflammation, which were present at all dose levels. There were no statistically

Table 12. Inhibition of cholinesterase activity in rats administered chlorpyrifos-methyl dermally for 28 days

Cholinesterase sample	Time	Cholinesterase activity (% inhibition)							
		Dose (mg/kg bw per day)							
		0		10		100		300	
		M	F	M	F	M	F	M	F
Red blood cells	Week 4	0	0	3*	7*	30*	29*	53*	43*
	Week 6	0	0	—	—	—	—	30*	27*
Brain	Week 4	0	0	1	6*	7*	14*	26	27*
	Week 6	0	0	—	—	—	—	10*	12*
Heart	Week 4	0	0	17*	19*	43*	58*	72*	72*
	Week 6	0	0	—	—	—	—	0	2

From Stebbins, Yano & Baker (2000)
* $P < 0.05$

identified body weight differences between any treated groups compared with their respective controls. At the end of 4 weeks, males given 100 or 300 mg/kg bw per day had a 7% and 13% lower body weight gain, respectively, compared with controls. Females given 100 mg/kg bw per day had a 29% lower body weight gain compared with controls, but body weight gain in 300 mg/kg bw per day females was comparable with that of controls. These minimal/inconsistent alterations in body weight were considered not to be adverse. All dose levels had food consumption comparable with their respective controls throughout the study. Dose-related decreases in plasma, red blood cells, and brain and heart cholinesterase activities occurred in males and females at all dose levels. These decreases were statistically significant at each dose level, with the exception of brain cholinesterase of males given 10 mg/kg bw per day (Table 12). There was evidence of recovery, almost complete for heart cholinesterase activity. There were no notable effects on haematology, clinical chemistry or urinalysis parameters.

Increased adrenal weight (20–40%) and vacuolation of the adrenal cortex were evident at necropsy in both sexes dosed at 300 mg/kg bw per day and in males dosed at 100 mg/kg bw per day. Increased vacuolation of the adrenal zona fasciculata and reticulata was seen in all animals at all dose levels, but not in controls; at 10 mg/kg bw per day, the grading was very slight, in mid-dose males, it was slight, and in mid-dose females and all top-dose animals, it was graded moderate. In recovery-phase animals, the grading had reduced to very slight. Taking account of the reversibility and severity, the adrenal findings at the low dose level are not considered to be adverse.

The NOAEL was 10 mg/kg bw per day, based on marked inhibition of heart cholinesterase activity and vacuolation of the adrenals at 100 mg/kg bw per day; the magnitude of these effects at 10 mg/kg bw per day is not considered adverse. There was no NOAEL for local effects (Stebbins, Yano & Baker, 2000).

(c) Inhalation route

Rats

Groups of Fischer 344 rats (10 of each sex) were exposed nose only to Reldan F (purity 96.8%; lot No. NB05272036) at levels of 0, 0.49, 3.7 or 18 parts per billion (ppb) for 6 h/day, 5 days/week, for 2 weeks. The atmosphere was generated by passing air over molten (~50 ºC) Reldan F; the mass median aerodynamic diameter was 0.68 μm. Five rats of each sex per group were terminated after

2 weeks; the remainder formed a 2-week recovery group. Clinical observations, body weights, food consumption and gross pathology were evaluated for all animals. In addition, haematology, clinical chemistry, urinalysis, organ weights and an extensive histopathological examination of tissues were evaluated in the exposure group rats only. There were no clinical signs other than those associated with confinement in nose-only exposure chambers. There were no notable effects on any measured parameter, including cholinesterase activities.

The no-observed-adverse-effect concentration (NOAEC) is 18 ppb (approximately 100 μg/m^3, equivalent to 0.027 mg/kg bw per day) (Stebbins & McGuirk, 2000).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Crj:CD-1 mice (96 of each sex per group; 52 scheduled for 78-week termination) were administered chlorpyrifos-methyl (purity 97.4%; batch No. AGR 219561) in the diet at concentrations of 0, 1, 5, 50 or 500 ppm for up to 18 months. Ten animals of each sex per group were terminated at weeks 26 and 52; urinalysis and measurement of haematological and clinical chemistry parameters were performed on these animals. An additional 10 animals of each sex per group were killed at weeks 26 and 52 for the measurement of non-fasted plasma, erythrocyte and brain cholinesterase activities using an automated procedure based on the method of Ellman et al. (1961). Groups of 10 animals of each sex were similarly investigated prior to necropsy at week 78. Weights of the brain, liver, adrenals, heart, kidneys and testes were recorded at necropsy (10 of each sex per group). A comprehensive list of tissues from animals killed at week 52 (0 and 500 ppm) and week 78 (all groups) and from all unscheduled deaths were subjected to histopathological examination. Histopathology was also performed on the lungs, liver, kidneys, adrenals and gross lesions from animals in intermediate dose groups killed at week 52.

Dietary analyses showed that the concentration, stability and homogeneity of the test material were within acceptable limits. Achieved mean intakes were 0, 0.08, 0.4, 4.4 and 44 mg/kg bw per day in males and 0, 0.08, 0.4, 3.9 and 41 mg/kg bw per day in females. No treatment-related effect on survival was seen. Survival of animals is considered to be adequate for the assessment of tumorigenicity. No clinical signs of toxicity were noted. Mean body weights of top-dose males were consistently lower than those of controls throughout the study period; values occasionally attained statistical significance. Mean body weights of top-dose females were lower than those of controls during the first months of the study and occasionally attained statistical significance. Urinalysis did not reveal any treatment-related findings. No treatment-related haematological changes were noted. Total plasma cholesterol concentrations were increased in top-dose animals of both sexes; values attained statistical significance in females at all time points and in males at week 26 only. Plasma ASAT and ALAT activities were significantly increased in top-dose females at week 52 (Table 13).

Plasma and erythrocyte cholinesterase activities were significantly reduced at 50 ppm and above at all time points. Brain cholinesterase activities were significantly decreased at the top dose level at all time points and at 50 ppm in week 52 females; the latter finding is not considered treatment related (Table 14), as it was not reproduced at 78 weeks, unlike the effect on red blood cells and on brain at 500 ppm. The high variation of control cholinesterase activity values is noted.

No treatment-related effects were seen on organ weight. Macroscopically, the incidence of pale liver was increased at the top dose level from week 26. Microscopically, the incidence of hepatic centrilobular fatty change was increased in both sexes at the top dose level; findings were apparent from week 52. The total incidence of diffuse hepatic fatty change was increased in females at all dose levels. This finding is of unclear toxicological significance in the absence of a dose–response relationship or similar findings in males. Renal tubular atrophy was noted with greater frequency in

Table 13. Clinical chemistry results in mice exposed to chlorpyrifos-methyl for 78 weeks

Parameter	Week	Males					Females				
		Dietary concentration (ppm)									
		0	1	5	50	500	0	1	5	50	500
Cholesterol (mg/dl)	26	114	115	166	103	159*	85	85	69	95	123*
	52	123	122	110	100	173	75	87	98	85	134*
	78	134	115	113	94	128	76	80	99	108	116**
ASAT (U/l)	52	42	53	47	60	63	38	52	59*	54	88**
	78	66	74	87	70	127	85	103	64	65	76
ALAT (U/l)	52	29	34	36	47	48	14	22	25	22	54**
	78	42	39	68	46	83	20	37	24	23	25

From Yoshida et al. (1988)
U, units; * $P < 0.05$; ** $P < 0.01$

Table 14. Cholinesterase activities in mice exposed to chlorpyrifos-methyl for 78 weeks

Cholinesterase sample	Week	Cholinesterase activity, U/ml (% of controls)									
		Males					Females				
		Dietary concentration (ppm)									
		0	1	5	50	500	0	1	5	50	500
Red blood cells	26	0.17	0.18	0.16	0.09**	0.01**	0.09	0.09	0.08	0.06**	0.01**
		(—)	(106)	(94)	(53)	(6)	(—)	(100)	(89)	(67)	(11)
	52	0.22	0.23	0.22	0.11**	0.01**	0.15	0.12	0.13	0.10**	0.01**
		(—)	(105)	(96)	(48)	(4)	(—)	(80)	(87)	(67)	(7)
	78	0.15	0.16	0.06	0.08*	0.01**	0.16	0.15	0.14	0.11**	0.02**
		(—)	(107)	(107)	(53)	(7)	(—)	(94)	(88)	(69)	(13)
Brain	26	0.32	0.33	0.35	0.32	0.15**	0.20	0.20	0.20	0.18	0.11**
		(—)	(103)	(109)	(100)	(47)	(—)	(100)	(100)	(90)	(55)
	52	0.60	0.61	0.62	0.54	0.27**	0.36	0.32	0.30	0.27**	0.18**
		(—)	(102)	(103)	(90)	(45)	(—)	(89)	(83)	(75)	(50)
	78	0.36	0.34	0.34	0.31*	0.13**	0.40	0.39	0.38	0.38	0.20**
		(—)	(94)	(94)	(86)	(36)	(—)	(98)	(95)	(95)	(50)

From Yoshida et al. (1988)
U, units; * $P < 0.05$; ** $P < 0.01$

males at 50 ppm and higher; the incidence of renal cortical cysts was increased in top-dose males. Both of these lesions are reported to be common findings in ageing CD-1 mice. Histopathological examination revealed cytoplasmic swelling of cells in the zona fasciculata of the adrenal cortex in both sexes; findings were more marked in males (Table 15). Amyloid change in a number of organs was apparent in a small number of animals of both sexes at 50 ppm and higher. The incidence of hepatocellular adenoma was marginally increased at the top dose level in both sexes; however, no clear dose–response relationship was apparent, and survival, particularly in males, was lower in controls. The incidence of hepatocellular carcinoma was comparable in all groups. No treatment-related effect on total tumour incidence was seen.

Table 15. Findings at necropsy in mice (n = 20 at week 52; n = 52 at week 78) exposed to chlorpyrifos-methyl for 78 weeks

Finding		Week	Incidence									
			Males					Females				
			Dietary concentration (ppm)									
			0	1	5	50	500	0	1	5	50	500
Survival (%)		*78*	*62*	*67*	*67*	*62*	*73*	*71*	*64*	*73*	*71*	*75*
Pale liver		52	1	1	0	2	3	0	0	0	1	3
		78	1	1	0	0	6	0	0	0	1	1
		Total	7	6	3	4	12	3	2	2	4	6
Centrilobular fatty change		52	5	4	5	6	18**	1	2	0	3	17*
		78	9	17	12	11	17	3	3	2	5	20**
		Total	15	21	17	18	39**	4	6	2	9	38**
Diffuse fatty change		Total	0	1	1	0	0	2	8*	9*	9*	8*
Renal tubular atrophy		78	16	23	19	23	29*	13	10	16	14	15
		Total	25	35	26	36**	40**	23	18	19	20	20
Renal cortical cyst		78	1	3	3	6	7*	5	6	5	8	2
		Total	3	5	5	8	11*	8	9	6	14	8
Adrenal cortex cell swelling		52	0	0	0	0	6*	0	0	0	0	0
		78	0	0	0	0	21*	0	0	0	0	3
		Total	0	0	0	0	28**	0	0	0	0	3
Hepatocellular adenoma		78	15	12	14	18	21	3	0	5	2	5
		Total	21	26	25	28	28	3	3	5	4	7
Total tumour incidence	Benign		32	36	38	41	39	21	15	27	23	24
	Malignant		24	19	31	24	24	21	23	21	27	20
	Neoplastic		45	49	55	53	52	38	34	42	40	38

From Yoshida et al. (1988)
* $P < 0.05$; ** $P < 0.01$

No evidence of carcinogenicity was seen in this study. A NOAEL of 50 ppm, equal to 3.9 mg/kg bw per day, can be determined for this study, based on decreased brain cholinesterase activity (>20%) in both sexes at 500 ppm (Yoshida et al., 1988).

Rats

In a 1974 (non-GLP) chronic toxicity study, CD rats (55 of each sex per group) were administered chlorpyrifos-methyl (purity 99.68%) at variable levels equivalent to 0, 0.03, 0.1, 1 or 3 mg/kg bw per day in the diet for up to 2 years. Sequential interim kills reduced the number of rats to 30 of each sex at week 52. A range of observations and investigations were performed during the study. Brain cholinesterase activity was measured in all rats at scheduled necropsy. Cholinesterase activities were assessed using the titrimetric method of Casterline & Williams (1967). Weights of nine organs were recorded at necropsy. Twenty-five tissues from the control and top dose groups were subjected to histopathological investigation.

Dietary analyses revealed acceptable concentration and stability of the test material. No treatment-related deaths or clinical signs were reported. No effects were seen on survival, food consumption, body weight or food conversion efficiency. Overall survival was 67–76% for males and 62–73% for females. Urinalysis did not reveal any treatment-related effects. Packed cell volume and haemoglobin concentration were significantly decreased in females at weeks 25 and 52; however, these effects are not considered to be clearly treatment related in the absence of similar findings at later time points. No treatment-related effects on clinical chemistry parameters were noted. Erythrocyte cholinesterase and plasma cholinesterase activities are noted to be variable but were consistently reduced at 3 mg/kg bw per day. No significant effects were seen on brain cholinesterase activity. Ophthalmoscopy did not reveal any treatment-related findings. Absolute and relative thyroid weights were significantly lower in 26-week interim kill females at 1 and 3 mg/kg bw per day and in 3 mg/kg bw per day females at week 52. The toxicological significance of this finding is unclear in the absence of microscopic correlates or similar findings at week 104. No treatment-related macroscopic or microscopic findings attributable to the test material were noted at necropsy. Tumour incidence was comparable in all treatment groups.

A NOAEL of 3 mg/kg bw per day can be determined for this study in the absence of any consistent, treatment-related effects. This study does not comply with modern standards owing to the small group size and the limited nature of the histopathological examinations. The high purity of the test material is also noted (Hunter et al., 1974).

In a 1991 combined chronic toxicity and carcinogenicity study, Fischer 344 rats (50 of each sex per group) were administered chlorpyrifos-methyl (purity ~95%; batch No. AGR 219561) in the diet at concentrations calculated to be equivalent to 0, 0.05, 0.1, 1 or 50 mg/kg bw per day for up to 104 weeks. An additional 10 rats of each sex per group were assigned for interim kill at week 52. Haematological and clinical chemistry parameters (10 of each sex per group) were measured in blood samples taken from the orbital sinus at weeks 26 and 52 (interim kill animals) and at weeks 78 and 104 (terminal kill animals). Plasma and erythrocyte cholinesterase activities were also measured, using an automated procedure based on the method of Ellman et al. (1961). Samples for urinalysis were collected at weeks 26 and 52 (interim kill animals) and weeks 78 and 104 (terminal kill). Brain cholinesterase activity was determined in half-brain homogenates at interim and terminal necropsy (10 of each sex per group). Weights of the adrenals, brain, heart, liver, kidneys and gonads were recorded at necropsy. A comprehensive list of organs were examined histopathologically.

Dietary analyses revealed satisfactory stability, homogeneity and achieved concentration of the test material. Terminal survival (60–72% in males and 64–86% in females) is considered adequate for the assessment of tumorigenicity. Group mean body weights were slightly (but significantly) lower in top-dose males from week 10 to termination. No consistent effects on body weight were seen at lower dose levels or in females. Food consumption in top-dose males was consistently lower than that of controls; however, values did not attain statistical significance.

Significant differences in red blood cell parameters and platelet counts were noted at 26 and 52 weeks in both sexes at the top dose level. With the exception of the red blood cell count in females, effects were not apparent after treatment for 78 weeks. No significant haematological effects were seen after 104 weeks (Table 16). Plasma cholesterol levels were consistently increased in top-dose females; values attained statistical significance at 52 and 104 weeks. Decreased serum enzyme (AP, ALAT, ASAT and creatine kinase) activities noted at the top dose level are not considered to be of toxicological significance.

Plasma cholinesterase activities were significantly decreased at 1 mg/kg bw per day and higher at all time points. Erythrocyte cholinesterase activities were decreased consistently at the top dose level; the findings at 0.1 and 1 mg/kg bw per day were inconsistent over time and without a clear

Table 16. Haematology and clinical chemistry findings in rats exposed to chlorpyrifos-methyl for 104 weeks

Parameter	Week	Males					Females				
		Dose (mg/kg bw per day)									
		0	0.05	0.1	1	50	0	0.05	0.1	1	50
Red blood cells (10^6/ml)	26	9.12	9.21	9.02	9.00	8.64*	8.04	8.16	8.14	8.09	7.78*
	52	8.90	8.90	8.85	8.85	8.60*	8.30	8.10	8.19	8.37	7.83*
	78	8.92	8.85	8.68	8.92	9.11	8.26	8.46	8.46	8.38	7.66*
	104	8.01	8.52	8.60	7.21	7.91	7.78	7.49	7.52	7.69	7.32
Haemoglobin (g/dl)	26	15.5	15.8	15.1	15.12	14.6*	14.8	15.0	14.9	14.7	14.3*
	52	14.3	14.2	14.2	14.1	13.8*	14.9	14.6	14.6	14.9	14.2*
	78	15.4	15.1	14.9	15.2	15.6	14.9	15.2	15.0	15.1	14.1
	104	14.7	15.5	15.3	13.1	14.0	14.7	14.3	14.3	14.5	14.0
Packed cell volume (%)	26	44.5	44.5	43.0	43.1*	41.7*	41.9	42.2	41.5	40.7*	39.8*
	52	44.3	43.5	43.4	43.4	42.4*	45.0	44.0	44.1	44.6	42.4*
	78	44.6	44.2	43.6	44.9	46.0	44.0	45.1	44.8	44.5	42.2
	104	42.5	44.8	44.4	38.0	40.8	40.9	39.5	39.7	40.8	40.0
Platelets (10^3/ml)	26	546	584	595	558	619*	556	589	589	572	606
	52	607	584	598	578	646*	505	513	505	517	530
	78	612	580	603	603	617	522	485	452	497	519
	104	513	527	554	489	491	476	446	457	477	502
Cholesterol (mg/dl)	26	74.8	71.0	71.7	76.7	78.4	115.4	114.9	106.3	109.5	127.8
	52	102.1	95.9	97.4	101.0	105.7	143.0	144.8	144.0	148.2	175.1*
	78	76.3	74.8	76.0	70.2	73.8	77.2	74.7	79.8	75.8	101.1
	104	135.9	189.9	179.3	195.3	167.5	128.4	124.1	125.2	130.5	222.4*

From Barna-Lloyd, Szabo & Davis (1991)
* $P < 0.05$

dose–response. Brain cholinesterase activities were significantly decreased at the top dose level only (Table 17).

Mean absolute and relative adrenal weights were significantly increased in top-dose animals of both sexes at interim and final kills. Mean relative liver weights were slightly (but significantly) increased at the top dose level in both sexes at the interim kill and in females only at the terminal kill. The toxicological significance of this finding is unclear in the absence of histological correlates. Mean testes and kidney weights were slightly increased at the terminal kill; kidney weights attained statistical significance in females only.

Treatment-related findings at interim necropsy were limited to slightly increased incidences of altered foci in the adrenal cortex zona fasciculata and Leydig cell hyperplasia in top-dose males. At terminal necropsy, the incidence of splenomegaly was increased in top-dose females; this finding is considered to be secondary to the higher incidence of large granular cell leukaemia ("Fischer rat leukaemia") in this group. Vacuolation of the adrenal cortex zona fasciculata was significantly increased in males at 1 mg/kg bw per day and higher (Table 18) and in females at the top dose level

Table 17. Cholinesterase activities and organ weights in rats exposed to chlorpyrifos-methyl for 104 weeks

Parameter	Week	Males					Females				
		Dose (mg/kg bw per day)									
		0	0.05	0.1	1	50	0	0.05	0.1	1	50
Erythrocyte cholinesterase, U/ml (% of control)	26	2.09	2.22	2.19	1.78	1.47	2.69	2.42	2.10*	2.12*	1.74*
		(—)	(106)	(105)	(85)	(70)	(—)	(90)	(78)	(79)	(65)
	52	2.03	1.95	1.97	1.82	1.25*	2.11	1.77	1.99	1.89	1.85
		(—)	(96)	(97)	(90)	(62)	(—)	(84)	(94)	(90)	(88)
	78	2.92	2.32	2.57	2.77	2.09	1.86	1.52	1.69	1.88	1.67
		(—)	(79)	(88)	(95)	(72)	(—)	(82)	(91)	(101)	(90)
	104	2.24	2.12	2.23	2.09	1.78*	2.15	2.19	2.21	2.10	1.74*
		(—)	(95)	(100)	(93)	(79)	(—)	(102)	(103)	(98)	(81)
Brain cholinesterase, U/g (% of control)	52	11.55	10.26	11.30	11.44	6.65*	11.82	11.93	12.19	12.08	7.59*
		(—)	(89)	(98)	(99)	(58)	(—)	(101)	(103)	(102)	(64)
	104	10.44	9.46	9.40	10.22	5.53*	10.25	10.7	10.99*	10.66	6.43*
		(—)	(91)	(90)	(98)	(53)	(—)	(105)	(107)	(104)	(63)
Organ weights at interim kill (% of body weight)											
Adrenal		0.010	0.011	0.011	0.011	0.019*	0.021	0.023	0.024	0.024	0.0938*
											0.0383[a]
Liver		2.542	2.443	2.453	2.478	2.678*	2.637	2.636	2.610	2.634	2.830*
Organ weights at final kill (% of body weight)											
Adrenal		0.017	0.017	0.019	0.111	0.035*	0.018	0.020	0.021	0.022	0.026*
					0.016[a]						
Liver		3.136	3.130	3.000	3.034	3.432	2.862	2.713	2.769	2.678	3.231*
Testes		1.670	1.561	1.553	1.293*	2.055*					
Kidney		0.829	0.965	0.874	0.810	0.870	0.719	0.740	0.741	0.724	0.794*

From Barna-Lloyd, Szabo & Davis (1991)
U, units; * $P < 0.05$
[a] Excluding one animal with phaeochromocytoma.

only; the findings were not graded for severity. Findings were reported to be consistent with lipid accumulation. The incidence in 1 mg/kg bw per day males is within the historical control range for the laboratory (0–42%). Increased incidences of lung microgranuloma and nasal foreign body reaction are thought to be secondary to the inhalation of food material and are not of toxicological significance. The significantly increased incidence of pituitary pars distalis adenoma in top-dose females was not clearly dose related and was stated to be within the historical control range for the rat strain (0–70%). The incidence of pituitary hyperplasia was also slightly increased in this group; however, no treatment-related effect was apparent for pituitary adenocarcinoma or for total pituitary tumours. The incidences of thyroid parafollicular cell hyperplasia and adenoma were slightly increased in top-dose animals of both sexes. The increased incidence of uterine endometrial polyps in top-dose females is within the historical control range for the rat strain (0–37%). The greater tumour burden in top-dose females is largely attributable to increased incidences of benign tumours, specifically pituitary adenoma and uterine endometrial polyp, as discussed above.

Table 18. Findings at necropsy in rats exposed to chlorpyrifos-methyl in the diet for 104 weeks

Finding		Incidence (no. of animals)									
		Males					Females				
		Dose (mg/kg bw per day)									
		0	0.05	0.1	1	50	0	0.05	0.1	1	50
		Interim sacrifice (/10)									
Adrenal vacuolation		0	0	0	0	10	0	0	0	0	10
Altered adrenal foci		0	1	1	1	2	1	1	0	1	1
Multifocal Leydig cell hyperplasia		3	0	1	0	7					
		Decedents and terminal sacrifice (/50)									
Splenomegaly		15	12	17	20	11	7	5	6	5	14
Spleen: large granular cell leukaemia		14	12	17	20	14	8	5	5	5	14
Adrenal vacuolation		3	5	9	11*	50*	2	2	1	0	49*
Lung microgranuloma		10	9	4	6	20*	4	2	1	1	1
Nasal foreign body reaction		9	1	2	3	18*	2	0	0	0	0
Pituitary	Cystic	6	2	1	5	4	16	19	13	11	13
	Hyperplastic	12	9	14	11	8	10	7	8	11	16
	Adenoma	7	17	12	17	8	13	14	27*	27*	23*
	Adenocarcinoma	1	1	1	0	1	6	6	2	3	3
	Total tumours	8	18	13	17	9	19	20	29	30	26
Thyroid parafollicular	Hyperplasia	6	4	1	1	10	6	0	0	3	12
	Adenoma	2	2	2	0	3	3	0	1	2	6
Endometrial polyp							9	7	11	8	18
Animals with tumours	Benign	49	49	48	50	50	25	23	34	35	36
	Malignant	20	22	22	24	21	19	12	10	10	20
	Total	50	50	50	50	50	37	30	39	40	44

From Barna-Lloyd, Szabo & Davis (1991)
* □ = 0.05

No evidence of tumorigenicity was seen in the rat following administration of chlorpyrifos-methyl for up to 2 years. A NOAEL of 1 mg/kg bw per day can be determined for this study, based on decreased brain cholinesterase activity, increased adrenal weights and associated histopathology at 50 mg/kg bw per day. Animals were fasted prior to termination; it is therefore possible that terminal cholinesterase inhibition was underestimated in this study. However, reassurance is gained from cholinesterase results in a previous 2-year rat study (Barna-Lloyd, Szabo & Davis, 1991).

A histopathology review panel performed a "blind" reading of the adrenal slides from the study of Barna-Lloyd, Szabo & Davis (1991). The review included a scoring for severity of vacuolation that was absent from the original study. The review panel concluded that the findings of adrenal vacuolation at 1 mg/kg bw per day and below were consistent with background findings and that the only dose producing clear effects was the top dose of 50 mg/kg bw per day (Table 19) (Bruner & Gopinath, 2000).

Table 19. Pathology review of adrenal slides in rats exposed to chlorpyrifos-methyl in the diet for 104 weeks

	Males					Females				
	Dose (mg/kg bw per day)									
	0	0.05	0.1	1	50	0	0.05	0.1	1	50
No. examined	50	50	50	50	50	50	50	50	50	50
Incidence (original)	3	5	9	11	50	2	2	1	0	49
Incidence (histopathology review panel)	13	10	14	22	50	10	10	6	7	50
- Minimal	6	5	9	14	0	5	8	4	7	0
- Mild	7	5	5	8	4	5	2	2	0	1
- Moderate	0	0	0	0	46*	0	0	0	0	49*

From Bruner & Gopinath (2000)
* $P < 0.05$

Dogs

In a poorly reported 1974 (non-GLP) study, Beagle dogs (seven of each sex per group) were administered Dowco 214 (purity 99.68%; batch No. 238-12-19) in the diet at concentrations equivalent to 0, 0.03, 0.1, 1 or 3 mg/kg bw per day for up to 104 weeks. Three dogs of each sex per group were killed after 6 months for histopathological examination and determination of brain cholinesterase activity. Plasma and erythrocyte cholinesterase activities were determined prior to treatment and at various points during the study according to the method of Casterline & Williams (1967). Limited haematological and clinical chemistry parameters were measured in dogs receiving 0, 1 and 3 mg/kg bw per day after 1, 3, 6, 12, 18 and 24 months. Urinalysis was also performed on these animals. Ophthalmoscopy and veterinary examinations were performed pretest and following treatment for 6, 12, 18 and 24 months. Weights of 15 organs were recorded at necropsy. Histopathology was performed on a comprehensive list of tissues from animals at 0, 1 and 3 mg/kg bw per day. Many results are presented as overall means, not split by sex.

Dietary analyses showed satisfactory achieved concentration and stability of test material. Weight gain was comparable in all groups from week 1 to week 26. Weight gain from week 0 to week 104 was lower in treated groups; slight mean weight loss was seen in top-dose males from weeks 26 to 40. Absolute body weight (males and females combined) was reduced by about 10% at the top dose level. Food and water consumption were not affected by treatment. The study report states that no treatment-related ophthalmological findings were found; however, observations are not reported. Neurological examinations revealed slightly increased abnormal reactions (reaction to sensation and segmental reflex) at the top dose level. No treatment-related effects on haematological, clinical chemistry or urinalysis parameters were noted at any time point.

A clear, dose-related decrease in plasma cholinesterase activity was apparent at 0.1 mg/kg bw per day and higher from week 4 (the first time point). Erythrocyte cholinesterase activity was clearly reduced at 1 mg/kg bw per day and higher from week 4; activity at 0.1 mg/kg bw per day was also reduced intermittently (Table 20). No clear time-related effects on plasma or erythrocyte cholinesterase activity were noted. No treatment-related effect was seen on mean brain cholinesterase activity; however, activities are noted by the present reviewers to be unusually low.

Mean absolute and relative liver weights were slightly increased at the top dose level at the interim and final necropsies. Values did not attain statistical significance and were stated to be within the normal range for the laboratory. Lower thymus weights at 1 and 3 mg/kg bw per day may reflect

Table 20. Plasma, erythrocyte (males and females combined) and brain cholinesterase activities in dogs exposed to chlorpyrifos-methyl in the diet for 2 years

Week	Cholinesterase activity, µmol/ml per minute (% of control)									
	Plasma					Erythrocyte				
	Dose (mg/kg bw per day)									
	0	0.03	0.1	1	3	0	0.03	0.1	1	3
Mean pretest	1.90	1.81	1.89	1.94	1.93	1.76	1.74	1.70	1.64	1.69
4	2.05	2.07	1.87	1.63	1.68	1.88	1.95	1.94	1.74	1.49
	(—)	(101)	(91)	(80)	(82)	(—)	(104)	(103)	(93)	(79)
26	1.50	1.28	1.16	1.00	0.90	1.49	1.32	1.16	0.87	0.75
	(—)	(85)	(77)	(67)	(60)	(—)	(89)	(78)	(58)	(50)
27[a]	1.62	1.31	1.25	1.06	0.97	1.25	1.10	0.99	0.87	0.74
	(—)	(81)	(77)	(65)	(60)	(—)	(88)	(79)	(70)	(59)
39[a]	1.41	1.45	1.36	1.07	1.06	1.37	1.28	1.21	1.21	0.94
	(—)	(103)	(96)	(76)	(75)	(—)	(93)	(88)	(88)	(69)
52[a]	1.58	1.50	1.40	1.24	0.94	1.28	1.36	1.37	1.19	0.95
	(—)	(95)	(89)	(78)	(59)	(—)	(106)	(107)	(93)	(74)
69[a]	1.62	1.54	1.48	1.23	1.00	1.49	1.54	1.65	1.32	1.29
	(—)	(95)	(91)	(76)	(62)	(—)	(103)	(111)	(89)	(87)
78[a]	1.74	1.82	1.65	1.21	1.09	1.52	1.41	1.47	1.18	0.70
	(—)	(105)	(95)	(70)	(63)	(—)	(93)	(97)	(78)	(46)
102[a]	2.15	1.87	2.05	1.36	1.19	2.18	2.15	2.05	1.93	1.51
	(—)	(87)	(95)	(63)	(55)	(—)	(99)	(94)	(89)	(69)
	Brain									
	Males					Females				
	Dose (mg/kg bw per day)									
	0	0.03	0.1	1	3	0	0.03	0.1	1	3
26[b]	0.025	0.023	0.017	0.020	0.023	0.022	0.020	0.017	0.021	0.019
	(—)	(92)	(68)	(80)	(92)	(—)	(91)	(77)	(95)	(86)
104[a]	0.018	0.019	0.024	0.018	0.023	0.020	0.021	0.025	0.017	0.019
	(—)	(106)	(133)	(100)	(128)	(—)	(105)	(125)	(85)	(95)

From Rivett et al. (1974)

[a] Four of each sex per dose level.

[b] Three of each sex per dose level.

the relatively small group size and the high normal variation for this parameter. No treatment-related histopathological findings were noted.

A NOAEL of 1 mg/kg bw per day can be determined for this study, based on decreased body weights in top-dose males. This study is deficient in a number of areas, specifically the limited nature of the investigations, low brain cholinesterase activity and limited reporting. The high purity of the test material used in this study is noted (Rivett et al., 1974).

2.4 Genotoxicity

Testing of the genotoxicity of chlorpyrifos-methyl has been performed in a wide range of assays. Many of the studies were performed in the 1980s and were not conducted according to GLP

or in compliance with current test guidelines. However, the overall database is considered adequate to conclude that chlorpyrifos-methyl is unlikely to be genotoxic.

Data on the genotoxicity of chlorpyrifos-methyl are summarized in Table 21.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a 1973 (non-GLP) study, groups of 10 male and 20 female Sprague-Dawley rats were fed Dowco 214 (batch No. 238-1219) in the diet for three generations at concentrations equivalent to 0, 1 or 3 mg/kg bw per day. The purity of the test material was not reported; however, other studies using the same batch have reported a purity of 99.68%. Exposure to the test material was continuous through the 9-week premating, mating, gestation and lactation periods. Following at least 9 weeks of exposure, the P_1, P_2 and P_3 animals were mated (one male to two females) to produce the first of two litters per generation (F_{1A}, F_{2A} and F_{3A}). Rats were mated 2 weeks after the completion of weaning to produce second litters (F_{1B}, F_{2B} and F_{3B}). Successive generations were derived from second litters. Litters were culled to 10 pups on day 4 postpartum. Plasma, erythrocyte and brain cholinesterase activities were measured in P_3 animals only (five of each sex) according to an automated procedure based on the method of Ellman et al. (1961). Comprehensive histopathological examination of P_3 animals (five of each sex from the control and 3 mg/kg bw per day groups) was performed.

Dietary homogeneity and stability of the test material were not reported. No clinical signs of toxicity or treatment-related deaths were noted during the study period. No treatment-related effects on body weight or food consumption were seen in parental animals of any generation. The fertility index of top-dose dams was slightly but consistently lower in each generation. No treatment-related effects on gestation length or pup survival indices were seen. Survival (days 4–21) of F_{3B} pups was low in all groups; this was attributed to the effects of diarrhoea in this generation (actual incidences were not reported). Body weights of treated F_{3B} pups were significantly lower than those of controls; however, there was no clear dose–response, and these findings are not clearly attributable to the effects of treatment and may be secondary to the diarrhoea reported in this generation. Comparable effects were not seen in F_{3A} pups (Table 22).

No treatment-related macroscopic or microscopic findings were noted. Plasma cholinesterase activity was significantly decreased in all treated P_3 groups. Erythrocyte cholinesterase activity was decreased in top-dose males and in both groups of treated females. No treatment-related effect on brain cholinesterase activity was seen (Table 23).

A NOAEL of 1 mg/kg bw per day can be determined for this study, based on slightly (but consistently) decreased fertility at the top dose level. This study is considered to have serious deficiencies, including the lack of purity and stability data, the use of only two dose levels, the lack of maternal toxicity at the high dose level and the limited nature of the histopathological and cholinesterase investigations (Thompson, Dyke & Warner, 1975).

Groups of 30 male and 30 female CD rats were fed diets containing variable levels of Reldan F (purity 96.8%; batch No. NB05272036) to give intakes of 0, 1, 3 or 10 mg/kg bw per day for approximately 10 weeks prior to mating and continuing through gestation (3 weeks) and lactation (3 weeks) for each of two generations. A comprehensive evaluation of male and female reproductive systems was conducted, including an evaluation of estrous cyclicity, gonadal function, mating performance, conception, gestation, parturition, lactation and weaning, as well as survival, growth and development of the offspring. Litters were culled to eight pups on day 4. Cholinesterase activity in

Table 21. Results of studies of genotoxicity with chlorpyrifos-methyl

Test substance (vehicle)	End-point	Test object	Concentration	Purity (%); batch	Result	Reference
In vitro						
Dowco 214 (DMSO)	Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	1, 10, 500, 500, 100, 2500, 5000, 10 000 µg/plate, ±S9	99.9; AGR 95563	Negative	DeGraff (1983)
Dowco 214 (DMSO)	Gene mutation (*HPRT*)	Chinese hamster ovary cells	5, 20, 40, 60,[a] 80, 100 µmol/l, ±S9	95.2; AGR 219561	Negative	Mendrala (1985)
Dowco 214 (DMSO)	Chromosomal aberration	Chinese hamster ovary cells	4, 12, 40[a] µg/ml, −S9 5,[a] 15, 50 µg/ml, +S9	95.2; AGR 219561	Negative −S9 Positive +S9	Gollapudi, Linscombe & Sinha (1985)
Dowco 214 (DMSO)	Unscheduled DNA synthesis	Rat primary hepatocytes	1, 3, 10, 30,[a] 100 µmol/l	95.2; AGR 219561	Negative	Mendrala & Dryzga (1985)
In vivo						
Dowco 214 (corn oil)	Micronucleus assay	CD-1 mouse bone marrow (gavage)	0, 146, 460, 1460 mg/kg bw	95.2; AGR 219561	Negative	Bruce, Gollapudi & Hinze (1985)
Reldan F (corn oil)	Unscheduled DNA synthesis	Sprague-Dawley rats (gavage)	0, 600, 2000 mg/kg bw	98.3; RMM 1710	Negative	Proudlock (1994)

DMSO, dimethyl sulfoxide; S9, 9000 × g supernatant from livers of rats

[a] Cytotoxicity.

Table 22. Fertility and litter parameters in a reproduction study in rats with chlorpyrifos-methyl

Parameter		Generation	Litter A			Litter B		
			Dose (mg/kg bw per day)					
			0	1	3	0	1	3
Fertility index (%)		F_1	95	100	85	85	95	79
		F_2	90	100	85	90	100	70
		F_3	100	100	80	100	95	80
Survival, days 4–21 (%)		F_1	93	90	95	77	81	75
		F_2	97	96	98	79	87	97
		F_3	79	76	80	47	65	35
Pup weight (g)	Day 0		6.1	6.0	6.3*	6.3	6.1*	6.2*
	Day 4	F_3	7.7	7.8	8.0	8.0	7.3*	6.4*
	Day 21		35.7	34.9	35.5	36.7	31.8*	33.5*

From Thompson, Dyke & Warner (1975)
* $P < 0.05$

Table 23. Cholinesterase activities in P_3 rats in a reproduction study with chlorpyrifos-methyl

Cholinesterase sample	Cholinesterase activity, µmol/ml per 3 min (% of controls)					
	Males			Females		
	Dose (mg/kg bw per day)					
	0	1	3	0	1	3
Plasma	2.3 (—)	1.5* (65)	1.1* (48)	7.4 (—)	3.0* (41)	2.0* (27)
Red blood cells	4.1 (—)	3.3 (80)	1.2* (29)	4.2 (—)	3.2* (76)	1.7* (40)
Brain	5.4 ± 0.5 (—)	8.0 (148)	7.5 (139)	7.1 ± 0.8 (—)	8.0 (113)	8.5 (120)

From Thompson, Dyke & Warner (1975)
* $P < 0.05$

plasma, erythrocytes, brain and heart was determined in five P_1 and five P_2 adults of each sex per group. Cholinesterase activities were also determined in 10 F_1 and 10 F_2 pups of each sex per group (taken from five litters) on postnatal day 1 and 5 F_1 and 5 F_2 pups of each sex per group (taken from the same litters as on postnatal day 1) on postnatal days 4 and 22. In-life observations, body weights, food consumption and litter data were evaluated. A gross necropsy of the P_1 adults and day 22 weanlings (three of each sex per litter) was conducted, with extensive histopathological examination of tissues. These postmortem evaluations included oocyte quantification and assessment of sperm count, motility and morphology in adults and gross pathology and organ weights in weanlings.

Adrenal weights and vacuolation of the zona fasciculata were increased in parental animals given 10 mg/kg bw per day and in females given 3 mg/kg bw per day. Red blood cell acetylcholinesterase activity was inhibited in a dose-dependent manner in adult rats of all sexes, generations and dose levels. The only other significant effect on adult acetylcholinesterase activity was a moderate inhibition (<20%) in brain (P_1 and P_2 males and females) and in heart (P_1 males only) at the highest dose level (Table 24). In general, pup acetylcholinesterase activity was unaffected by treatment, with the exception of effects on red blood cell acetylcholinesterase activity in the high-dose pups on postnatal day 1. There were no adverse effects of chlorpyrifos-methyl on any parameter of reproductive performance, pup survival, growth or development (Table 25).

Table 24. Cholinesterase results from a rat multigeneration study with chlorpyrifos-methyl

Age	Dose (mg/kg bw per day)	Cholinesterase activity (% of control)					
		Males			Females		
		Red blood cells	Brain	Heart	Red blood cells	Brain	Heart
P_1 adults	1	79.7	104.3	97.7	82.7*	97.1	102.4
	3	39.5*	100.1	84.7	32.7*	96.4	97.8
	10	19.3*	86.7*	75.4*	6.0*	81.4*	78.1
P_1 pups day 1	1	78.9	97.4	88.2	101.9	99.8	91.4
	3	91.6	93.5	91.4	110.0	92.7	100.3
	10	63.5*	93.5	88.5	76.6	91.3	93.2
P_1 pups day 4	1	102.9	103.3	94.7	106.3	96.2	107.5
	3	82.2	90.0	94.4	102.8	95.2	111.7
	10	70.5	101.7	94.0	78.5	96.0	110.7
P_1 pups day 22	1	112.0	104.8	98.1	105.8	91.5	99.6
	3	108.6	100.3	99.4	95.9	96.3	97.8
	10	128.6*	102.5	95.7	120.5	90.8	86.7
P_2 adults	1	82.9	97.3	100.3	83.5*	101.3	91.9
	3	30.7*	94.9	95.0	37.8*	100.3	85.9
	10	9.3*	88.1*	86.4	6.8*	90.4*	77.1*
P_2 pups day 1	1	103.6	91.8	95.7	113.6	91.9	104.1
	3	111.9	92.3	100.4	113.9	90.5	101.0
	10	74.2*	93.5	102.0	84.7	97.8	100.7
P_2 pups day 4	1	126.1	101.8	92.7	112.7	96.7	100.2
	3	100.4	105.2	99.9	89.9	108.3	100.9
	10	94.7	106.4	109.0	74.7	107.4	97.2
P_2 pups day 22	1	124.8	104.9	91.3	102.7	100.3	105.7
	3	104.7	101.9	101.1	93.8	100.8	106.4
	10	119.6	103.7	92.1	103.7	100.9	109.2

From Carney et al. (2002)
* $P < 0.05$

The NOAEL for reproduction is 10 mg/kg bw per day, the highest dose tested. The NOAEL for parental toxicity is 1 mg/kg bw per day, based on adrenal changes at 3 and 10 mg/kg bw per day. The NOAEL for pup development is 10 mg/kg bw per day, the highest dose tested (Carney et al., 2002).

(b) Developmental toxicity

Rats

Mated female Sprague-Dawley rats (≥20 per group) were gavaged with Dowco 214 (purity not specified, in corn oil) at 0, 50, 100 or 200 mg/kg bw per day on days 6–15 of gestation. Blood samples were taken on day 16 from control and 200 mg/kg bw per day animals for the analysis of cholinesterase activity using an automated method based on Ellman et al. (1961). Animals were terminated on day 21 of gestation. Approximately half of the fetuses were examined by serial sectioning, and half were examined for skeletal variations following staining with Alizarin Red. Cholinesterase activity was measured in control and top-dose fetal homogenates ($n = 5$).

Table 25. Reproductive parameters in rats exposed to chlorpyrifos-methyl

Parameter	F_1 mating				F_2 mating			
	Dose (mg/kg bw per day)							
	0	1	3	10	0	1	3	10
Number of males	30	30	30	30	29	30	30	30
Number of females	30	30	30	30	30	30	30	30
Time to mating (days)	2.4 ± 1.6	2.2 ± 1.8	2.5 ± 2.5	2.3 ± 0.9	2.7 ± 1.7	2.2 ± 1.1	2.3 ± 1.3	2.4 ± 1.8
Male mating index (%)	100	100	100	96.7	100	96.7	100	100
Female mating index (%)	100	100	100	96.7	100	96.7	100	100
Male fertility index (%)	83.3	83.3	83.3	86.7	82.8	66.7	86.7	80.0
Female fertility index (%)	83.3	83.3	83.3	86.7	80.0	66.7	86.7	80.0
Gestation length (days)	21.6 ± 0.6	21.5 ± 0.5	21.6 ± 0.5	21.8 ± 0.5	21.6 ± 0.6	21.5 ± 0.5	21.5 ± 0.5	21.9 ± 0.3
Pregnant dams delivering (%)	100	100	100	100	100	100	100	100
Gestation survival index (%)	99.0	98.5	97.7	99.7	99.4	99.0	98.6	99.0
Day 1 survival (%)	96.5	98.7	99.1	99.4	98.7	98.9	99.7	98.7
Days 1–4 survival (%)	89.7	91.9	93.1	93.1	91.3	89.8	94.1	90.9
Days 4–21 survival (%)	95.3	97.4	98.5	97.1	98.4	98.7	98.5	97.9
Litter size day 21	7.2 ± 1.3	7.6 ± 1.0	7.9 ± 0.3	7.7 ± 0.8	7.5 ± 1.7	7.8 ± 0.7	7.6 ± 1.1	7.6 ± 0.9
Mean pup weight day 21 (g)	53.4	52.2	53.9	54.6	50.1	46.6	49.9	51.6

From Carney et al. (2002)

No clinical signs of toxicity were reported in this study, although erythrocyte and plasma cholinesterase activities were essentially zero on day 16 (Table 26). Maternal body weight was slightly (but significantly) decreased at 200 mg/kg bw per day. Small increases in the incidence of omphalocoele, renal pelvis dilatation, subcutaneous oedema, lumbar ribs or spurs and delayed sternebral ossification were noted in the fetuses of treated groups. Fetal cholinesterase activity was significantly reduced at 200 mg/kg bw per day (Table 26).

No evidence of teratogenicity was noted in this study. In the absence of any measurements of brain or erythrocyte cholinesterase activities in intermediate dose groups, a NOAEL cannot be determined for this study (Schwetz et al., 1973).

In a range-finding study, mated female CD rats (10 per dose level) were gavaged with Reldan (purity 96.9%; batch No. EK900512002/RMM1710) in corn oil at 0, 12.5, 50 or 200 mg/kg bw per day from days 6 to 15 of gestation. Salivation was observed in most rats on day 7 and subsequently at 200 mg/kg bw per day. At 50 mg/kg bw per day, about half the animals salivated after dosing on

Table 26. Maternal and fetal findings in pregnant rats exposed to chlorpyrifos-methyl by gavage

Finding		Dose (mg/kg bw per day)			
		0	50	100	200
Maternal findings					
Maternal body weight, day 16 (g)		320	319	328	307*
Cholinesterase activity (μmol/ml per minute)	Plasma	2.846[a]			0.012*[b]
	Red blood cells	1.226[a]			0.000*[b]
Fetal findings					
Omphalocoele, % fetal incidence (% litter incidence)		—	—	—	0.7 (4)
Renal pelvis dilatation, % fetal incidence (% litter incidence)		2 (10)	—	1 (8)	7 (20)
Subcutaneous oedema, % fetal incidence (% litter incidence)		19 (45)	—	29 (67)	32 (68)
Lumbar ribs/spurs, % fetal incidence (% litter incidence)		0.6 (3)	0 (0)	2 (8)	5 (20)
Delayed sternebral ossification, % fetal incidence (% litter incidence)		0.6 (3)	5 (26)	11 (38)	8 (28)
Brain cholinesterase activity (μmol/ml per minute)		1.038			0.670**

From Schwetz et al. (1973)
* $P \leq 0.05$; ** $P < 0.01$
[a] $n = 2$.
[b] $n = 5$.

occasional days after three or more doses; there was no indication of increased frequency as the dosing period progressed, and there was no salivation reported on the last day of dosing. Blood samples for the measurement of cholinesterase activity were taken 2 h following the final dose; brains were removed 1 day after the final dose. Fetuses were examined for gross external malformations. Details of the cholinesterase results are in Table 27 below. No fetal abnormalities were reported.

In the main study, which was designed to meet United States Environmental Protection Agency guidelines, mated female CD rats (30 per dose level) were gavaged with Reldan (purity 96.9%; batch No. EK900512002/RMM1710) in corn oil at 0, 1, 12.5 or 50 mg/kg bw per day from days 6 to 15 of gestation. Blood samples were taken immediately prior to termination on day 20 for the measurement of cholinesterase activity according to the method of Ellman et al. (1961). Brain cholinesterase activity was determined from half-brain homogenates. Approximately half of the fetuses were examined for visceral abnormality by freehand serial sectioning and half for skeletal abnormalities by Alizarin Red staining. In this study, blood samples for the measurement of cholinesterase activity were taken at termination on day 16, 2 h following the final dose; brains were removed 4 days after the final dose.

No effects on body weight or food consumption were seen in dams. Salivation immediately post-dosing was noted in a small number of animals at 50 mg/kg bw per day; no further signs of toxicity were observed. Plasma cholinesterase activity was significantly decreased in dams at 50 mg/kg bw per day, and erythrocyte cholinesterase activity was significantly decreased at 12.5 mg/kg bw per day and higher. Brain cholinesterase activity was slightly (but significantly) decreased at 50 mg/kg bw per day (Table 27). No treatment-related findings were noted at gross necropsy of dams.

The combined incidence of microphthalmia/anophthalmia and the incidences of interventricular septal defect, renal pelvis dilatation and reduced ossification of the cervical vertebral arches were increased in fetuses at 50 mg/kg bw per day (Table 28); however, the increases are marginal and are not clearly a direct result of treatment. The number of normal sternebrae was significantly higher in the top dose group.

Table 27. Cholinesterase activity in pregnant rats exposed to chlorpyrifos-methyl by gavage

Cholinesterase sample	Cholinesterase activity, µmol/ml per minute (% of control)							
	Dose (mg/kg bw per day)							
	0		1	12.5		50		200
	Main	Range-finding	Main	Main	Range-finding	Main	Range-finding	Range-finding
Plasma	1.60	1.46	1.47	1.48	0.33	1.40**	0.18	0.10
	(—)	(—)	(92)	(93)	(23)	(88)	(12)	(7)
Red blood cells	1.59	1.37	1.60	1.06**	0.46	0.85**	0.48	0.17
	(—)	(—)	(101)	(67)	(34)	(53)	(35)	(12)
Brain	8.01	4.67	8.67	7.93	4.18*	7.09**	3.54**	2.11**
	(—)	(—)	(108)	(99)	(90)	(89)	(76)	(45)

From Bryson (1992)
* $P < 0.05$; ** $P < 0.01$

Samples for the measurement of cholinesterase activity in this study were taken at termination (i.e. 5 days following the final administration of test material). Considerable underestimation of cholinesterase inhibition is therefore likely. In the range-finding study, samples were taken 2 h following termination at day 16. Cholinesterase values from the range-finding study are therefore considered to be more representative and are noted by the Meeting to be lower than those from the main study.

Owing to the marginal nature of the brain cholinesterase effects at 12.5 mg/kg bw per day in the range-finding study, an overall maternal NOAEL of 1 mg/kg bw per day can be determined. There was no evidence of teratogenicity, and the NOAEL for teratogenicity is 50 mg/kg bw per day, the highest dose tested. Indications of fetotoxicity (developmental delay) were seen at the top dose level, and a developmental NOAEL of 12.5 mg/kg bw per day can therefore be determined for this study (Bryson, 1992).

Rabbits

In a 1976 (non-GLP) study, mated female Japan White rabbits (10 per dose level) were gavaged with chlorpyrifos-methyl (dissolved in acetone and mixed with corn oil) at 4, 8 or 16 mg/kg bw per day from days 6 to 18 of gestation. Rabbits were terminated on day 29 of gestation, and pups were examined for visceral and skeletal abnormalities. The dose levels used in this study were based on the results of a range-finding study in which decreased plasma cholinesterase activities of 23.3% and 32.3% were seen at 16 and 32 mg/kg bw per day, respectively, following 14 days of administration of chlorpyrifos-methyl.

Mean body weight, mean body weight gain and mean food consumption were lower at 8 and 16 mg/kg bw per day. The numbers of fetuses were also lower at these dose levels. However, values reflected lower corpora lutea and implantation numbers (Table 29) and therefore, taken with the absence of a dose–response, are not considered to be treatment related. No increase in preimplantation or postimplantation loss was seen at these dose levels. No gross external or visceral malformations were noted in any fetus. No treatment-related effect on the incidence of skeletal findings was reported.

Table 28. Fetal findings in rats exposed to chlorpyrifos-methyl during pregnancy

Fetal observation	Dose (mg/kg bw per day)			
	0	1	12.5	50
Anophthalmia/microphthalmia, fetal (litter) incidence	—	—	—	1 (1)
Interventricular septal defect, fetal (litter) incidence	—	1 (1)	—	2 (2)
Renal pelvis dilatation, fetal (litter) incidence	1 (1)	—	—	3 (2)
Reduced ossification – cervical vertebral arches, fetal (litter) incidence	—	—	—	2 (2)
Normal sternebrae (% of total)	40.8	34.3	45.1	58.9*
Abnormal sternebrae (% of total)	59.2	65.7	54.9	41.1*

From Bryson (2002)
* $P < 0.05$

Only 10 pregnant animals per dose level were used in this study, whereas the current OECD guideline recommends the use of 20 animals, with a minimum of 16. Even so, the study is considered adequate to assess the teratogenic potential of chlorpyrifos-methyl in rabbits.

No evidence of teratogenicity was noted in this study. A maternal NOAEL of 4 mg/kg bw per day can be determined for this study, based on decreased body weight, body weight gain and food consumption. No assessment of cholinesterase activity was carried out in the main study; inhibition of plasma cholinesterase activity was noted at 16 mg/kg bw per day in the range-finding study. A developmental NOAEL of 16 mg/kg bw per day, the highest dose tested, can be determined for this study (Asai et al., 1976).

2.6 Special studies

(a) Data on metabolites

Data on the primary plant metabolite of chlorpyrifos-methyl, TCP, were considered by the 1999 JMPR during the review of chlorpyrifos (Annex 1, reference *86*). The acute oral toxicity of TCP is moderate, with LD_{50}s in the range of 380–1000 mg/kg bw. In repeated-dose toxicity studies, the liver was the main target organ, with the lowest NOAEL of 12 mg/kg bw per day in the dog. TCP was not genotoxic in vitro or in vivo. There were no developmental effects in rats at dose levels up to 150 mg/kg bw per day, but in rabbits, there were increased incidences of abnormalities, primarily dilatation of the cerebral ventricles and hydrocephaly at 100 mg/kg bw per day and above. The NOAEL was 25 mg/kg bw per day.

(b) Effects on the central nervous system

No acute or repeated-dose neurotoxicity studies or developmental neurotoxicity studies have been performed with chlorpyrifos-methyl.

Acute delayed neuropathy

Domestic hens (six per group) were gavaged with a single dose of Reldan (purity and batch details not given) in corn oil at 0, 3240, 3645, 4050, 4455 or 4860 mg/kg bw and observed for 21 days. A positive control group was dosed with tri-*ortho*-cresyl phosphate (TOCP) at 500 mg/kg bw. Survivors from the 4455 mg/kg bw group and two additional animals were redosed on day 21 following intramuscular injection of atropine (10 mg/kg bw) and pralidoxime methiodide (50 mg/kg bw).

Table 29. Maternal and fetal findings in rabbits dosed with chlorpyrifos-methyl

Observation	Time	Dose (mg/kg bw per day)			
		0	4	8	16
Body weight (kg)	Day 18	3.33 ± 0.08	3.54 ± 0.09	3.11 ± 0.07	3.04 ± 0.06
Body weight gain (kg)	Days 6–18	0.23	0.22	0.16	0.16
Food consumption (g/day)	Days 6–18	141.4	134.7	112.4	113.3
Number of corpora lutea		8.5 ± 0.34	9.0 ± 0.39	6.7 ± 0.42	7.2 ± 0.78
Number of implantations		8.3 ± 0.39	8.5 ± 0.42	6.3 ± 0.39	6.9 ± 0.90
Litter size		7.6 ± 0.47	8.4 ± 0.42	6.1 ± 0.45	6.5 ± 0.87

From Asai et al. (1976)

Deaths occurred in TOCP- and Reldan-treated groups. Lethargy was observed in hens dosed with Reldan and also in the control group, suggesting a vehicle effect. Ataxia, ranging from slight loss of coordination to difficulty in walking, was noted in all birds dosed with TOCP. No consistent effect was seen on body weight. Significant neuropathological changes, including spinal axonal degeneration and mid-brain neuronal and perineuronal vacuolation, were observed in the three TOCP-dosed decedents. Hens dosed with Reldan were not examined histopathologically owing to the absence of clinical signs.

The acute oral LD_{50} of Reldan in the hen was found to be 4455–4860 mg/kg bw per day. No evidence of neurotoxicity was seen in hens administered chlorpyrifos-methyl; however, histopathological investigations were not performed, there was no forced activity and NTE activity was not measured (Ross, Burroughs & Roberts, 1975).

In a 1979 (non-GLP) study, "adult" White Leghorn hens (10 per group) were administered a single dose of chlorpyrifos-methyl (purity 95.9%; batch No. AGR-142688) by capsule at 0, 2500 or 5000 mg/kg bw. All hens had been administered atropine sulfate at 30 mg/kg bw by capsule 30 min previously. A positive control group was similarly treated using tri-*ortho*-tolyl phosphate (TOTP) at 250 mg/kg bw. Hens were observed for 21 days, and body weights were recorded weekly. Sciatic nerve and cervical, thoracic and lumbar sections of the spinal cord were examined histopathologically following staining with H&E and Luxol Blue. The sciatic nerve was additionally investigated using Bodian's silver stain.

No deaths occurred during the study period. No clinical signs were noted in controls or at 2500 mg/kg bw. Ataxia and unsteady gait were noted in four hens of the high dose group from day 2. All hens appeared normal by day 7, suggesting incomplete protection by atropine. Hens administered TOTP exhibited progressive signs of neurotoxicity (altered locomotion and loss of coordination) from day 11. Weights of hens dosed with chlorpyrifos-methyl were significantly less than those of controls at day 7, but were comparable at day 14. Body weights of TOTP-dosed hens were significantly lower than those of controls at day 21. Histopathological examination revealed effects in all positive control birds. Spinal findings consisted of vacuolation and demyelination of the cord, with swollen and fragmented axons. Observations in the sciatic nerve consisted of vacuolation and distension of the neurolemma and axonal fragmentation. A number of findings were associated with local inflammatory responses. Similar histopathological findings were noted in six birds dosed with chlorpyrifos-methyl at 5000 mg/kg bw (Table 30). One bird showed evidence of neurotoxicity in both the spinal cord and sciatic nerve, and one additional bird showed evidence of spinal change only. Findings in other birds in this dose group were apparent in either the sciatic nerve or the spinal cord and were described as equivocal. One hen from the 2500 mg/kg bw group had focal alterations of the

Table 30. Neuropathy findings in hen given a single dose of chlorpyrifos-methyl

Test material	Dose level (mg/kg bw)	Deaths	Clinical signs		Histopathology	
			Number	Duration	Spine	Nerve
Chlorpyrifos-methyl	0	—	—	—	—	—
	2500	—	—	—	—	1+
	5000	—	4/10	Days 2–6	2+/2±	1+/2±
TOTP	250	—	10/10	Days 11–21	10+	10+

From Clarke, Warner & Johnstone (1975)
+, positive findings; ±, equivocal findings

sciatic nerve, vacuolation/distension of the neurolemma sheath, granular/fragmentation of axons and increased sarcolemma cells or mononuclear inflammatory cells.

Equivocal histological evidence of neurotoxicity was seen in this study following a supralethal dose of chlorpyrifos-methyl, with only a few test animals showing effects. This study does not comply fully with modern guidelines, as forced motor activity was not assessed, no histopathology was performed on the brain or tibial nerve and NTE activity was not measured (Clarke, Warner & Johnstone, 1975).

Repeated-dose delayed neuropathy

In a 1983 range-finding study, 30-week-old White Leghorn hens (five per group) were gavaged with chlorpyrifos-methyl (purity 96.2%; batch No. AGR 209075) in corn oil at 0, 50, 100, 250, 500, 750 or 1000 mg/kg bw per day, 5 times weekly, for 4 weeks. At necropsy, brain, spinal cord, sciatic nerve and/or tibial nerve were examined histopathologically. Two top-dose birds were found dead on days 15 and 24. Egg production ceased after 1 week at 500 mg/kg bw per day and above. Slight to severe ataxia was noted in two birds at 750 mg/kg bw per day (days 16–17, days 16–22). Signs of mild central nervous system depression described as a "tendency to sleep when undisturbed" were noted in three top-dose birds. Slight ataxia was noted in all top-dose survivors on day 17, with apparent recovery by the following day. "Severe ataxia" was noted in one bird on day 22 prior to its death on day 24. "Early paralysis/inability to walk" was noted in an additional top-dose bird on day 23. Body weights of top-dose birds were significantly lower than those of controls from day 14. Loss of body fat was noted in this group at necropsy. No adverse findings were noted in nervous tissue (Barna-Lloyd et al., 1984).

In a 1984 (non-GLP) study, groups of 10 White Leghorn hens were gavaged with chlorpyrifos-methyl (purity 96.2%; batch No. AGR 209075) in corn oil at 0, 5, 50 or 500 mg/kg bw per day, 5 days/week, for 13 weeks. Additional groups were similarly administered TOCP at 10 or 30 mg/kg bw per day. Routine observations included forced activity on a 30-degree ramp. High-dose positive controls were killed after treatment for 6 weeks as a result of marked signs of neurotoxicity. Selected nervous system tissues were removed at necropsy and examined histopathologically, as detailed in Table 31.

Mean body weights of the top-dose chlorpyrifos-methyl group (from day 3) and high-dose TOCP group (from day 31) were significantly lower than those of controls. Egg production was significantly reduced in the top-dose TOCP and top-dose chlorpyrifos-methyl groups. No clinical signs of neurotoxicity were noted in hens dosed with chlorpyrifos-methyl. Slight ataxia was noted in two hens dosed with TOCP at 10 mg/kg bw per day following forced exercise. Clinical signs in high-dose TOCP hens ranged from severe ataxia to severe paralysis.

Table 31. Histopathology techniques for the repeated-dose delayed neuropathy study with chlorpyrifos

Tissue	Staining procedure[a]		
	1	2	3
Cerebellum, brainstem and optic lobes (TS at middle of optic lobe)	✓	✓	✓
Cerebellum and brainstem (TS at cerebellar peduncles)	✓	—	—
Caudal medulla (TS)	✓	—	—
Mid-cervical, thoracic and lumbosacral spinal cord (TS and LS)	✓	✓	✓
Sciatic and tibial nerve (TS and LS)	✓	✓	✓

From Barna-Lloyd et al. (1984)
LS, longitudinal section; TS, transverse section
[a] 1, H&E; 2, Luxol Fast Blue/Periodic acid-Schiff (myelin specific); 3, Sevier-Munger silver (axon specific).

Necropsy revealed macroscopic findings in high-dose chlorpyrifos-methyl and high-dose TOCP groups as detailed in Table 32. Microscopic examination of peripheral and central nervous system tissues revealed findings characteristic of delayed neurotoxicity in TOCP groups; findings were more marked at the high dose level. Histopathological findings were apparently increased in the chlorpyrifos-methyl dose groups; however, these findings were of low grade, showed no significant dose–response, were stated to be similar to background changes reported in the literature and were not considered by the examining pathologist to represent evidence of delayed neurotoxicity (Table 32). NTE inhibition was not assessed in this study (Barna-Lloyd et al., 1984).

NTE inhibition

An in vitro investigation of the potency of chlorpyrifos-methyl oxon to inhibit NTE was performed in 2001. Brain microsomal homogenates were prepared from White Leghorn hens. Assays for NTE and acetylcholinesterase activities were performed to determine the inhibition rate constant (k_i) and the concentration producing 50% inhibition of activity (IC_{50}) for test compounds. Test compounds were chlorpyrifos oxon, chlorpyrifos-methyl oxon and mipafox (a compound reported to produce organophosphate-induced delayed neuropathy in humans).

The results showed that chlorpyrifos-oxon and chlorpyrifos-methyl oxon were very similar in their inhibitory properties, showing a far higher potency against acetylcholinesterase than NTE, whereas mipafox was approximately equipotent (Table 33). The results indicate that chlorpyrifos-methyl is unlikely to induce delayed neurotoxicity at sublethal doses (Richardson & Kropp, 2001).

3. Observations in humans

3.1 Studies in volunteers

Dowco 214 was applied daily to the non-abraded dorsal and abdominal skin of three human volunteers at different dose levels (10, 25 or 50 mg/kg bw per day). A number of applications were made under occlusive dressings for 12 h. Plasma and erythrocyte cholinesterase activities were measured according to the method of Nabb & Whitfield (1967). Rabbits were similarly exposed for comparison purposes. No clinical signs of toxicity were noted in any subject. Plasma cholinesterase activity was slightly depressed in the volunteer administered daily dermal exposures of 10 mg/kg bw per day from day 10. Effects were maximal at 12 days; further applications did not depress values further. Cholinesterase activity had returned to normal by day 25. Following daily exposure to 25 mg/kg bw, plasma cholinesterase activity had decreased by 47.5% at day 4. Recovery was

Table 32. Findings at necropsy in the repeated-dose delayed neuropathy study with chlorpyrifos-methyl

Findings	Incidence (no. of animals out of 10 examined)					
	Control	Chlorpyrifos-methyl			TOCP	
		Dose (mg/kg bw per day)				
		5	50	500	10	30
Shrunken comb	0	0	0	6	0	3
Muscle atrophy	0	0	0	4	0	2
Loss of body fat	0	0	0	2	0	7
Cervical spinal cord						
- Axonal degeneration (dorsal funiculi)	—	—	—	—	1a, 3b, 2c	4b, 2c, 4d
- Axonal degeneration (lateral funiculi)	—	—	—	—	—	4b, 3c, 3d
- Focal gliosis	2a	5a	3a	4a	2a, 4b	6b, 2c, 2d
Thoracic spinal cord						
- Axonal degeneration	—	—	—	2a	1a, 4b, 1c	2b, 2c, 4d, 2e
- Focal gliosis	1a	5a	7a, 1b	6a	4a, 3b	5b, 1c, 2d, 2e
Lumbosacral spinal cord						
- Axonal degeneration	—	—	1a	—	1a, 5b, 2d	5d, 5e
- Focal gliosis	—	1a, 1b	2a, 1b	3a	5b	4b, 3c, 3e
Peripheral nerve						
- Fibre degeneration	—	—	—	1a	2a	1a, 2b, 2c, 2d
Cerebellar peduncle						
- Axonal degeneration	—	1a	—	1a	4a, 1c, 1d	1b, 1c, 1d, 7e
- Focal gliosis	—	—	2a	2a	1a, 1b	1b, 2c, 7d
Caudal medulla						
- Axonal degeneration	—	—	—	—	3a, 5b	5d, 5e
- Focal gliosis	—	—	—	—	2a, 1b	5b, 5c

From Barna-Lloyd et al. (1984)
a, very slight; b, slight; c, slight to moderate; d, moderate; e, moderate to severe

seen by day 28. No effect on plasma cholinesterase activity was seen following a single application of 50 mg/kg bw. Erythrocyte cholinesterase activity was not affected in any volunteer. Effects on rabbits were more severe, with almost complete inhibition of plasma cholinesterase activity at 10 and 25 mg/kg bw per day. Significant inhibition was also noted following a single application of 50 mg/kg bw. Inhibition of erythrocyte cholinesterase activity (90–95%) was seen at 10 and 25 mg/kg bw in the rabbit; marked inhibition was also noted at 50 mg/kg bw. The results of this study suggest that humans are relatively insensitive to the chlorpyrifos-methyl-induced inhibition of plasma and erythrocyte cholinesterase activity following dermal exposure. It is unclear whether this is due to lower penetration through the skin or a lower susceptibility to cholinesterase inhibition, or a combination of both (Killian, Edwards & Pennington, 1971).

In a 1975 (non-GLP) study, healthy male volunteers (4–5 per dose level) were administered capsules containing chlorpyrifos-methyl of unspecified purity at 0 (placebo control), 0.03 or 0.1 mg/

Table 33. Inhibition data for acetylcholinesterase and NTE from hen brain preparations incubated with three organophosphorus compounds

Inhibitor	k_i (per µmol/l per minute)		20-min IC_{50} (µmol/l)		RIP
	AChE	NTE	AChE	NTE	
Chlorpyrifos oxon	17.8 ± 0.3	0.0993 ± 0.0049	0.001 95 ± 0.000 03	0.349 ± 0.017	179 ± 9
Chlorpyrifos-methyl oxon	10.9 ± 0.1	0.0582 ± 0.0013	0.003 18 ± 0.000 03	0.595 ± 0.013	187 ± 4
Mipafox	0.004 29 ± 0.000 01	0.004 98 ± 0.000 06	8.08 ± 0.02	6.96 ± 0.08	0.861 ± 0.011

From Richardson & Kropp (2001)
AChE, acetylcholinesterase; RIP, relative inhibitory potency = $[k_i(\text{AChE})/k_i(\text{NTE})] = [IC_{50}(\text{NTE})/IC_{50}(\text{AChE})]$

kg bw per day for 28 days. Urinalysis, haematology and clinical chemistry investigations were performed pretest and weekly during the study. Erythrocyte and plasma cholinesterase activities were measured pretest and twice weekly during the study period according to the method of Nabb & Whitfield (1967). Volunteers signed informed consent forms and were monitored and interviewed twice weekly to assess signs and symptoms of toxicity; particular attention was given to cholinergic symptoms.

The purity and stability of the test material were not reported. A number of volunteers occasionally failed to take their doses; however, this is not considered to have affected the overall integrity of the study. Urinalysis, haematology and clinical chemistry investigations did not reveal any treatment-related effects. No effects were seen on the pulse rate or blood pressure of volunteers. Erythrocyte and plasma cholinesterase activities were not affected by administration of chlorpyrifos-methyl. The study report states that no signs or symptoms attributable to chlorpyrifos-methyl toxicity were noted. A NOAEL of 0.1 mg/kg bw per day can be determined for this study in the absence of any effects on cholinesterase activity (Coulston, Rosenblum & Griffin, 1975).

In a 1975 study, three male and three female volunteers were administered capsules of chlorpyrifos-methyl (purity 99.2%) in corn oil at 0.1 mg/kg bw per day for 21 days and at 0.3 mg/kg bw per day for the following 14 days. Following a 28-day recovery period, the same volunteers were dosed at 0.2 mg/kg bw per day for 21 days. Four control subjects (two of each sex) were administered placebo capsules. Erythrocyte and plasma cholinesterase activities were determined pretest and at intervals throughout the study period according to the method of Levine, Scheidt & Nelson (1965), a DTNB-based procedure based on that of Ellman et al. (1961). Selected haematological, urinalysis and clinical chemistry parameters were also assessed. No reference to informed consent was made in the study report.

No adverse effects on blood pressure, pulse rate, pupil size, light reflex, eye accommodation, chest sound, muscle tone, knee jerk or tongue tremor were reported. No treatment-related effects on haematological, clinical chemistry or urinalysis parameters were noted.

Significant interindividual and intraindividual variations in plasma and erythrocyte cholinesterase activities were noted. Treatment-related decreases in plasma cholinesterase activity were reported in volunteers following administration of chlorpyrifos-methyl at 0.2 and 0.3 mg/kg bw per day; no effects were seen at 0.1 mg/kg bw per day (Table 34). No consistent effects were seen on erythrocyte cholinesterase activity at any dose level in this study. Increased cholinesterase activity in volunteer CS was attributed to “withdrawal from birth-control medication”.

Table 34. Cholinesterase activities in human volunteers receiving chlorpyrifos-methyl

Group	Subject (sex)	Cholinesterase activity, μmol/ml per 3 min (% of control)							
		Pretest mean	0.1 mg/kg bw per day		0.3 mg/kg bw per day		Re-covery	0.2 mg/kg bw per day		Recovery
			Low[a]	Term[b]	Low	Term	Term	Low	Term	
Plasma cholinesterase										
Control	PW (F)	10.65	9.88	10.56	9.93	9.93	10.12[c]	9.66	9.66	
		(—)	(93)	(99)	(94)	(94)	(95)	(91)	(91)	
	CM (F)	8.79	7.59	8.50	8.01	9.20	9.18	8.02	8.59	
		(—)	(86)	(97)	(91)	(105)	(104)	(91)	(98)	
	CB (M)	13.69	11.80	13.05	12.63	13.71	12.78	12.77	13.84	
		(—)	(86)	(95)	(92)	(100)	(93)	(93)	(101)	
	PS (M)	16.18	15.35	16.23	14.00	16.83	17.20	16.51	17.03	
		(—)	(95)	(100)	(87)	(104)	(106)	(102)	(105)	
Test	CS[d] (F)	7.88	7.38	9.03	8.28	8.28	8.06	7.34	7.34	—
		(—)	(94)	(115)	(105)	(105)	(102)	(93)	(93)	
	JC (F)	10.55	9.75	9.75	8.40	8.40	10.35	9.38	9.38	9.18
		(—)	(92)	(92)	(80)	(80)	(98)	(89)	(89)	(87)
	BW (F)	11.38	10.56	10.56	9.63	9.95	11.02	7.77	7.77	11.82
		(—)	(93)	(93)	(85)	(87)	(97)	(68)	(68)	(104)
	RP (M)	14.16	13.36	14.45	13.01	13.17	14.80	14.34	14.66	—
		(—)	(94)	(102)	(92)	(93)	(105)	(101)	(104)	
	MG (M)	15.11	13.97	14.45	12.43	12.91	14.80	14.37	14.37	—
		(—)	(92)	(96)	(82)	(85)	(98)	(95)	(95)	
	KH (M)	12.35	11.42	12.80	9.34	9.20	12.14	8.00	8.00	9.04
		(—)	(92)	(104)	(76)	(74)	(98)	(65)	(65)	(73)
Erythrocyte cholinesterase										
Control	PW (F)	31.05	30.26	31.70	24.62	24.62	26.37[a]	30.05	30.05	
		(—)	(97)	(102)	(79)	(79)	(85)	(97)	(97)	
	CM (F)	29.48	28.45	29.75	26.15	28.58	29.52	29.87	30.51	
		(—)	(97)	(101)	(88)	(97)	(100)	(101)	(103)	
	CB (M)	35.74	33.34	35.66	32.76	32.76	32.53	27.50	35.28	
		(—)	(93)	(98)	(92)	(92)	(91)	(77)	(99)	
	PS (M)	28.94	26.39	29.75	24.46	26.55	27.74	27.90	27.90	
		(—)	(91)	(100)	(85)	(92)	(96)	(96)	(96)	

Table 34 (contd)

Group	Subject (sex)	Cholinesterase activity, μmol/ml per 3 min (% of control)								
		Pretest mean	0.1 mg/kg bw per day		0.3 mg/kg bw per day		Re-covery	0.2 mg/kg bw per day		Recovery
			Low[a]	Term[b]	Low	Term	Term	Low	Term	
Test	CS[b] (F)	35.89	30.13	39.07	30.16	30.55	35.45	34.46	34.70	—
		(—)	(84)	(109)	(84)	(85)	(99)	(96)	(97)	
	JC (F)	39.36	37.66	40.63	33.43	34.63	38.89	38.40	42.30	—
		(—)	(96)	(103)	(85)	(88)	(99)	(98)	(107)	
	BW (F)	37.55	34.48	39.34	31.72	35.29	36.09	37.15	37.15	29.29
		(—)	(92)	(105)	(84)	(94)	(96)	(99)	(99)	(78)
	RP (M)	29.24	26.78	31.70	25.99	25.99	27.74	28.70	28.70	30.74
		(—)	(92)	(108)	(89)	(89)	(95)	(98)	(98)	(105)
	MG (M)	33.10	29.79	33.86	28.58	28.58	31.47	31.48	31.48	—
		(—)	(90)	(102)	(86)	(86)	(95)	(95)	(95)	
	KH (M)	32.31	30.26	35.04	27.67	27.67	30.96	26.77	32.50	31.65
		(—)	(94)	(108)	(86)	(86)	(96)	(83)	(101)	(98)

From Chmiel (1975)
F, female; M, male
[a] Lowest value for treatment period.
[b] Value at termination of treatment period.
[c] Results for previous time point.
[d] See text.

A NOAEL of 0.3 mg/kg bw per day can be determined for this study in the absence of any biologically significant effects on erythrocyte cholinesterase inhibition (Chmiel, 1975).

In a double-blind, randomized, placebo-controlled study of the effects of single oral doses of the closely related and more potent cholinesterase inhibitor, chlorpyrifos (purity 99.8%), groups of six fasted men and six fasted women aged 18–55 received doses of 0, 0.5, 1 or 2 mg/kg bw in lactose powder. The study was conducted in two phases separated by 14 days. The volunteers were dosed with 0, 0.5 or 1 mg/kg bw in the first phase, and the results were assessed before administration of 0 or 2 mg/kg bw in the second phase. Blood samples were collected 10 and 0 h before treatment and 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144 and 168 h after treatment and analysed for erythrocyte cholinesterase activity and chlorpyrifos and its metabolites. In addition, all urine voided from 48 h before dosing to 168 h after dosing was collected at 12 or 6 h intervals and analysed for chlorpyrifos and its metabolites. Haematology, clinical chemistry, urinalysis and a brief physical examination were performed at completion of the study. Blood and urine were collected to determine each volunteer's paraoxonase status and the concentrations of chlorpyrifos and its metabolites, but these data were not reported. The volunteers were screened for general health according to set criteria and instructed to refrain from alcohol, strenuous exercise and prescription medications before and during the study. The doses were taken by capsule after an overnight fast. The health status of subjects was monitored closely; vital signs (blood pressure, pulse, respiration and temperature) were assessed before dosing and 1, 2, 4, 8, 12, 24, 48 and 168 h after treatment. The subjects were questioned about their well-being at each sampling time, and the symptoms were evaluated clinically. The subjects were aware of the signs and symptoms of cholinergic toxicity and were instructed to inform the study physician of any adverse

Table 35. Mean erythrocyte cholinesterase activity in volunteers given single oral doses of chlorpyrifos

Dose (mg/kg bw)	Mean erythrocyte cholinesterase activity as % of mean baseline value (±SD)							
	Time in relation to dosing (h)							
	−10	0	4	8	12	24	36	48
Women								
0.0[a]	99.5 (2.1)	100.5 (2.1)	98.3 (2.0)	102.1 (2.4)	100.7 (1.8)	99.7 (3.9)	98.4 (1.7)	100.2 (2.3)
0.0[b]	99.2 (4.4)	100.8 (4.4)	102.5 (5.9)	103.1 (5.4)	98.4 (3.2)	99.4 (3.7)	94.6 (5.3)	97.7 (5.1)
0.5	101.2 (6.7)	98.8 (6.7)	96.3 (6.5)	98.1 (2.1)	95.8 (3.9)	97.0 (3.6)	94.7 (4.7)	97.1 (4.1)
1	98.2 (0.9)	101.8 (0.9)	104.6 (4.2)	100.8 (3.5)	98.9 (3.7)	100.9 (5.6)	96.0 (4.1)	98.9 (2.1)
2	99.8 (1.8)	100.2 (1.8)	100.3 (3.2)	96.6 (6.81)	91.1 (9.7)	95.2 (10.6)	95.9 (7.4)	94.5 (7.7)
Men								
0.0[a]	100.6 (1.8)	99.4 (1.8)	99.1 (2.2)	99.6 (4.6)	99.8 (4.3)	98.4 (4.3)	97.6 (2.8)	98.2 (3.1)
0.0[b]	96.1 (5.1)	103.9 (5.1)	101.6 (4.0)	102.5 (5.2)	102.9 (5.8)	102.6 (2.3)	98.2 (3.8)	95.9 (6.8)
0.5	98.7 (1.1)	101.3 (1.1)	102.0 (3.0)	103.4 (4.9)	99.7 (3.0)	101.9 (3.3)	98.1 (3.0)	92.8 (4.7)
1	98.6 (1.9)	101.4 (1.9)	104.5 (7.8)	101.1 (4.2)	101.1 (4.7)	101.7 (4.7)	98.5 (4.6)	92.1 (2.0)
2	101.2 (2.3)	98.8 (2.3)	99.2 (3.1)	98.9 (3.5)	98.7 (4.0)	99.2 (2.6)	99.5 (5.1)	98.4 (3.1)

From Kisicki et al. (1999)
[a] Phase 2 control.
[b] Phase 1 control.

effects. The subjects were unaware of their treatment, and the signs and symptoms were assessed and treated by a physician who was also unaware of the treatment status of the subject.

There were no significant deviations from the study protocol. One male control in phase 1 and one woman receiving 2 mg/kg bw did not provide a complete series of blood and urine samples. Treatment had no effect on general health or on clinical chemical parameters measured 7 days after dosing. The only treatment-related effect was found in the woman who withdrew from the study, who had decreased erythrocyte cholinesterase activity when compared with her pretreatment values at most sampling times, with 98.4% of the pretreatment value at 4 h after dosing, 77% at 8 h, 72% at 12 h, 74% at 24 h, 81% at 36 h and 80% at 48 h (Table 35). When the data for this subject are removed from the analysis, the mean for women receiving 2 mg/kg bw is indistinguishable from the value for concurrent controls (Table 35). The NOAEL for clinical signs or symptoms was thus the highest dose tested, and the NOAEL for inhibition of erythrocyte cholinesterase activity was 1 mg/kg bw on the basis of significant inhibition in 1 of 12 subjects (Kisicki et al., 1999).

3.2 Workplace monitoring

Chlorpyrifos-methyl is produced in a single plant alongside other materials, including chlorpyrifos. A specific occupational hygiene monitoring and health surveillance programme was designed with regard to the manufacture and packaging of chlorpyrifos-methyl (Reldan) in the United Kingdom. Baseline examinations, involving physical, haematological and clinical chemistry investigations, were undertaken in 1980 prior to production. Plasma and erythrocyte cholinesterase activities were measured on three separate occasions before potential exposure. The mean of these estimations was used as each employee's baseline pre-exposure cholinesterase level. Exposure to chlorpyrifos-methyl (and intermediates) during manufacture was designed to be minimized by the use of engineering controls and personal protective equipment. Measured airborne concentrations of chlorpyrifos-methyl were typically less than 10% of those recommended in the production plant's internal occupational hygiene guide.

Plasma and erythrocyte cholinesterase activities were subsequently measured in all employees potentially exposed to chlorpyrifos-methyl. Results were recorded and compared with individual baseline values. Data were further analysed by shift and job type. Significant drops in cholinesterase activity of either individuals or groups resulted in modified work practices and investigation by the occupational health team. In accordance with company guidelines, employees were to be suspended from work if plasma cholinesterase activities were reduced by 80%. A depression of this extent was not encountered during the 15-year manufacturing period.

In 1994, a review and analysis of the health records of all employees currently (104) or previously engaged (114) in the manufacturing plant since 1980 were undertaken. The total data represented approximately 716 person-years. Conclusions from the analysis of these data revealed no significant health problems in the group studied when compared with colleagues on the same site unexposed to organophosphate insecticides. In addition, no documented cases of organophosphate poisoning or severe cholinesterase depression were found.

Data collected from 1991 to 1993 are stated to reveal a small but consistent drop in plasma and erythrocyte cholinesterase activities. The notifier suggests that exposure is probably via skin absorption and reports that this finding is not obvious unless individual data are pooled and compared with a suitable control group. No increases in morbidity, mortality or the incidence of neurological disorders in the study population over a 15-year period were seen when compared with a comparative control population (Dow, 2008).

More recent workplace monitoring data on chlorpyrifos-methyl have not been compiled.

Comments

Biochemical aspects

Chlorpyrifos-methyl is rapidly and extensively absorbed in rats given a single oral dose at 16 or 30 mg/kg bw. Excretion was rapid (largely within 24 h) and primarily in the urine. Urinary metabolites were identified as the glucuronide conjugate of TCP (68.6%), the desmethyl metabolite *O*-methyl-*O*-(3,5,6-trichloropyridyl) phosphorothioate (17.8%) and free TCP (13.8%). Although these results were reported very briefly, they are broadly consistent with data for the closely related compound chlorpyrifos (Annex 1, reference *86*). The fate of the phosphorothioate moiety was not investigated.

Toxicological data

Chlorpyrifos-methyl is of low acute toxicity when administered orally, dermally (LD_{50}s >2000 mg/kg bw) or by inhalation (LC_{50} >0.67 mg/l). Chlorpyrifos-methyl is a slight, transient irritant to skin and eye and has been found to produce skin sensitization in a Magnusson & Kligman maximization test, but not in a Buehler test.

Short-term studies of toxicity identified decreased cholinesterase activity and adrenal vacuolation as the most sensitive indicators of toxicity caused by chlorpyrifos-methyl. Studies did not show any consistent time-related progression in the inhibition of plasma or erythrocyte cholinesterase activity with repeated or prolonged administration of chlorpyrifos-methyl, suggesting that inhibition reaches a "steady state" relatively rapidly. There was evidence of significant but not complete recovery of cholinesterase activities after 2 or more weeks. In the 28-day study in mice, the NOAEL was 10 ppm, equal to 1.3 mg/kg bw per day, on the basis of reduced brain cholinesterase activity and vacuolation of the zona fasciculata of the adrenals. The same end-points were the basis for the NOAEL of 1 mg/kg bw per day in the 90-day study in rats. Decreased brain cholinesterase activity, decreased body weight gain, clinical chemistry and haematological findings were noted at the highest dose of 50 mg/kg bw per day in a 90-day study in dogs, with a NOAEL of 10 mg/kg bw per day.

No evidence of toxicity, including brain cholinesterase activity, was reported in a 6-month study in Rhesus monkeys given doses of up to 5 mg/kg bw per day.

The potential genotoxicity of chlorpyrifos-methyl has been investigated in an adequate battery of tests in vitro and in vivo. No evidence of mutagenicity was noted; however, chlorpyrifos-methyl was found to be clastogenic in Chinese hamster ovary cells in the presence of metabolic activation. Studies in vivo on micronucleus formation in bone marrow and on unscheduled DNA synthesis gave negative results.

The Meeting concluded that chlorpyrifos-methyl is unlikely to be genotoxic.

No evidence of carcinogenicity was seen in long-term studies of toxicity/carcinogenicity with chlorpyrifos-methyl in rats or mice. Adrenal pathology (vacuolation of the adrenal cortex zona fasciculata consistent with lipid accumulation) was noted in rats and mice. Having considered the outcome of a pathology review by a group that re-examined the slides of adrenal tissues obtained in the study in rats, the Meeting concluded that the findings at 1 mg/kg bw per day were not adverse. Decreased brain cholinesterase activity was found to be a consistent and sensitive indicator of chronic toxicity caused by chlorpyrifos-methyl. The inhibition of cholinesterase activity by chlorpyrifos-methyl seen in the long-term studies did not increase with duration of dosing. The NOAEL was 1 mg/kg bw per day in rats and 3.9 mg/kg bw per day in mice. Toxicity in a limited 2-year study in dogs was limited to reduced body weight gain at the highest dose of 3 mg/kg bw per day, with a NOAEL of 1 mg/kg bw per day.

The Meeting concluded that chlorpyrifos-methyl is not carcinogenic.

Marginal effects on fertility were seen at the highest dose of 3 mg/kg bw per day in an early three-generation study in rats; the NOAEL was 1 mg/kg bw per day. A subsequent, more extensive, two-generation study in rats found no effects on reproduction or pup development at 10 mg/kg bw per day; the NOAEL for parental toxicity was 1 mg/kg bw per day on the basis of findings in the adrenal gland. In an initial study of developmental toxicity in rats, there was no indication of teratogenicity at 200 mg/kg bw per day. Indications of delayed fetal development were seen at all doses (50 mg/kg bw per day and above), but without a clear dose–response relationship. In a range-finding study of developmental toxicity in rats, there was no indication of teratogenicity at 200 mg/kg bw per day, a dose producing salivation immediately after the second and subsequent doses and significant inhibition of cholinesterase activity. At 12.5 mg/kg bw per day, there was slight inhibition (10%) of brain cholinesterase activity 1 day after the final dose. The NOAEL for maternal toxicity was considered to be 1 mg/kg bw per day. The Meeting considered that the salivation was unlikely to be a result of systemic toxicity, as it occurred immediately after dosing, whereas the C_{max} was at 5 h, and there was evidence that chlorpyrifos-methyl tasted unpleasant at high concentrations. In a full study of developmental toxicity in rats, the NOAEL for maternal toxicity (brain cholinesterase activity 4 days after the final dose) and pup development (overall rate of anomalies) was 12.5 mg/kg bw per day, with a NOAEL for teratogenicity of 50 mg/kg bw per day, the highest dose tested. The only study of developmental toxicity in rabbits given chlorpyrifos-methyl was not performed to modern standards, but was considered adequate to assess the potential for teratogenicity. The NOAEL for maternal toxicity was 4 mg/kg bw per day on the basis of reductions in body weight gain and food consumption. The NOAEL for teratogenicity and fetal developmental toxicity was 16 mg/kg bw per day, the highest dose tested.

The Meeting concluded that chlorpyrifos-methyl caused developmental toxicity only at doses that were maternally toxic, but that it was not teratogenic.

The primary plant and mammalian metabolite of chlorpyrifos-methyl, TCP, was considered by the 1999 JMPR during the review of chlorpyrifos (Annex 1, references *86* and *88*). The acute oral toxicity of TCP is moderate, with LD_{50}s in the range of 380–1000 mg/kg bw. In studies of toxicity with repeated doses, the liver was the main target organ, with the lowest NOAEL of 12 mg/kg bw per day being identified in a study in dogs. TCP was not genotoxic in vitro or in vivo. There were no

developmental effects at doses of up to 150 mg/kg bw per day in rats, but rabbits showed increased incidences of abnormalities, primarily dilatation of the cerebral ventricles and hydrocephaly at 100 mg/kg bw per day and above, and the NOAEL was 25 mg/kg bw per day.

Some histopathological evidence of neuropathy was noted in hens given a single potentially lethal dose of chlorpyrifos-methyl at 5000 mg/kg bw. Equivocal histopathological findings noted in a short-term study of delayed neurotoxicity were considered to be similar to background findings and not consistent with delayed neuropathy. No assessment of NTE activity was made in the studies of neurotoxicity, but a study in vitro showed that chloryrifos-methyl oxon had a potency for inhibiting cholinesterase activity that was more than 100-fold that of NTE. This study also showed that chlorpyrifos-methyl oxon was less potent than chlorpyrifos oxon as an inhibitor of brain cholinesterase activity in hens.

The Meeting concluded that chlorpyrifos-methyl was unlikely to produce delayed neuropathy in the absence of very severe cholinergic toxicity.

In two studies in human volunteers exposed orally to chlorpyrifos-methyl for 21 or 28 days, there were no adverse findings concerning clinical signs, clinical chemistry or cholinesterase activity. The NOAEL was 0.3 mg/kg bw per day, the highest dose tested, over 21 days. A single oral dose of (the closely related compound) chlorpyrifos of up to 1 mg/kg bw did not significantly inhibit erythrocyte cholinesterase activity in human volunteers. The studies in human volunteers were considered to have been performed according to contemporary ethical standards.

There were no reports of adverse effects in production plant workers.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.01 mg/kg bw based on the NOAEL of 1 mg/kg bw per day, identified on the basis of inhibition of brain acetylcholinesterase activity and adrenal vacuolation in the 2-year study of toxicity and carcinogenicity in rats and with a safety factor of 100. This value is supported by the NOAEL of 1 mg/kg bw per day for inhibition of parental brain acetylcholinesterase activity in the multigeneration study of reproductive toxicity in rats and by the NOAEL of 1 mg/kg bw per day for inhibition of maternal brain acetylcholinesterase activity in the study of developmental toxicity in rats. The Meeting did not consider changes observed in the adrenals of rats given a dose of 1 mg/kg bw per day in the 2-year study to be treatment related, a conclusion that is consistent with that of the pathology review group. Limited studies in human volunteers, while not of sufficient quality (e.g. too few subjects, limited duration of treatment and the fact that no assessment of the adrenals was possible) to support their use in the derivation of an ADI, provide no basis for concern that the proposed ADI would not be adequately protective. In a number of studies, erythrocyte acetylcholinesterase activity was more sensitive than brain acetylcholinesterase activity to inhibition by chlorpyrifos-methyl. However, the Meeting noted that after oral administration, the sensitivity of heart acetylcholinesterase activity to inhibition by chlorpyrifos-methyl was similar to that of brain acetylcholinesterase. It was further noted that the differential sensitivity of acetylcholinesterase was the same as that observed with the close structural analogue chlorpyrifos (Marable et al., 2007). In vivo, the sensitivity of the enzyme in peripheral neuronal tissue is similar to that in the brain, whereas the enzyme in erythrocytes is more sensitive. The Meeting therefore concluded that inhibition of brain acetylcholinesterase activity, not erythrocyte acetylcholinesterase activity, was the appropriate end-point for use in the risk assessment of chlorpyrifos-methyl.

The Meeting established an ARfD of 0.1 mg/kg bw based on the NOAEL of 1 mg/kg bw, identified on the basis of the absence of inhibition of erythrocyte acetylcholinesterase activity in a single-dose study in human volunteers given the closely related compound chlorpyrifos, and with a safety factor of

10. The Meeting discussed whether an ARfD was necessary for chlorpyrifos-methyl, given the absence of any clear indications of systemic toxicity after single exposures. In the absence of adequate single-dose studies with extensive investigations of cholinesterase activity and clinical signs, the Meeting considered that it was not able to discount the possibility that chlorpyrifos-methyl could produce acute effects. The Meeting considered basing the ARfD on the repeated-dose study in human volunteers given chlorpyrifos-methyl, in which an overall NOAEL of 0.3 mg/kg bw per day was identified. It was noted that this was somewhat inconsistent with the higher NOAEL of 1 mg/kg bw in a single-dose study in humans given the closely related, but more potent, compound chlorpyrifos. Having considered data on the kinetics and acetylcholinesterase inhibition characteristics of chlorpyrifos and chlorpyrifos-methyl, the Meeting concluded that, although likely to be conservative, it was appropriate to use data from the single-dose study in humans given chlorpyrifos to establish the ARfD for chlorpyrifos-methyl. No other potentially acute effect that might serve as the basis for derivation of an ARfD was identified in studies in experimental animals.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	50 ppm, equal to 3.9 mg/kg bw per day	500 ppm, equal to 41 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 41 mg/kg bw per day[b]	—
Rat	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	1 mg/kg bw per day	50 mg/kg bw per day
		Carcinogenicity	50 mg/kg bw per day[b]	—
	Multigeneration study of reproductive toxicity[a]	Reproductive toxicity	10 mg/kg bw per day[b]	—
		Parental toxicity	1 mg/kg bw per day	3 mg/kg bw per day
		Offspring toxicity	10 mg/kg bw per day[b]	—
	Developmental toxicity[c]	Maternal toxicity	1 mg/kg bw per day	12.5 mg/kg bw per day
		Embryo/fetotoxicity	12.5 mg/kg bw per day	50 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	4 mg/kg bw per day	12–16 mg/kg bw per day
		Embryo/fetotoxicity	16 mg/kg bw per day[b]	—
Dog	Ninety-day study of toxicity	Toxicity	10 mg/kg bw per day	50 mg/kg bw per day
	Two-year study of toxicity[a]	Toxicity	1 mg/kg bw per day	3 mg/kg bw per day
Rhesus monkey	Twenty-six-week study of toxicity[c]	Toxicity	5 mg/kg bw per day[b]	—
Humans	Twenty-eight-day study of toxicity[d]	Toxicity	0.2 mg/kg bw per day[b]	—
	Twenty-one-day study of toxicity[d]	Toxicity	0.3 mg/kg bw per day[b]	—
	Single-dose study of toxicity with chlorpyrifos[d]	Toxicity	1 mg/kg bw[b]	—

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

Estimate of acceptable daily intake for humans

0–0.01 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 chlorpyrifos-methyl

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rats: rapid and extensive, >80%
Dermal absorption	Low: <5%, concentrated and diluted, rat epidermis in vitro
Distribution	Widely distributed
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid and almost complete, within 72 h, mainly via urine (83–85%), after a single dose
Metabolism in animals	Extensively metabolized; demethylation, hydrolysis, conjugation, oxidative desulfuration
Toxicologically significant compounds (animals, plants and the environment)	Parent and oxon
Acute toxicity	
Rat, LD_{50}, oral	2814 mg/kg bw
Rat, LD_{50}, dermal	>2000 mg/kg bw
Rat, LC_{50}, inhalation	>0.67 mg/l air (nose only)
Rabbit, dermal irritation	Slight, transient irritant
Rabbit, ocular irritation	Slight, transient irritant
Guinea-pig, dermal sensitization	Negative results in Buehler test; positive results in Magnusson & Kligman maximization test
Short-term studies of toxicity	
Target/critical effect	Inhibition of acetylcholinesterase activity, adrenal vacuolation
Lowest relevant oral NOAEL	1 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	10 mg/kg bw per day (systemic)
Lowest relevant inhalation NOAEC	18 ppb (approximately 100 µg/m^3)
Genotoxicity	
	No genotoxic potential in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Inhibition of acetylcholinesterase activity, adrenal vacuolation
Lowest relevant NOAEL	1 mg/kg bw per day
Carcinogenicity	Not carcinogenic
Reproductive toxicity	
Reproduction target/critical effect	Not toxic to reproduction
Lowest relevant reproductive NOAEL	10 mg/kg bw per day
Developmental target/critical effect	Not teratogenic; delayed fetal development, slight increase in abnormalities at maternally toxic doses
Lowest relevant developmental NOAEL	12.5 mg/kg bw per day (rat)

Neurotoxicity/delayed neurotoxicity	
	Histopathological indications of neuropathy at 5000 mg/kg bw; no indications of delayed neuropathy at 500 mg/kg bw per day for 13 weeks; very weak inhibitor of NTE in vitro
Other toxicological studies	
Studies in human volunteers	No adverse effects at doses of up to 0.3 mg/kg bw per day for 21 days
Single-dose study in human volunteers given chlorpyrifos	No adverse effects at doses of up to 1 mg/kg bw
Medical data	
	No adverse effects in production plant workers

Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Rat, 2 years, dietary	100
		Rat, reproductive toxicity	
		Rat, developmental toxicity	
		Dog, 2 years	
ARfD	0.1 mg/kg bw	Single-dose study in human volunteers given chlorpyrifos	10

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CYCLOXYDIM

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Explanation

Cycloxydim is the International Organization for Standardization (ISO)–approved name for (5*RS*)-2-[(*EZ*)-1-(ethoxyimino)butyl]-3-hydroxy-5-[(3*RS*)-thian-3-yl]cyclohex-2-en-1-one (International Union of Pure and Applied Chemistry [IUPAC]). The Chemical Abstracts Service (CAS) chemical name for cycloxydim is 2-[1-(ethoxyimino)butyl]-3-hydroxy-5-(tetrahydro-2H-thiopyran-3-yl)-

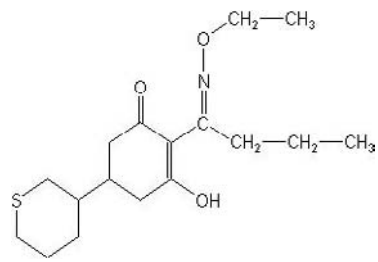
2-cyclohexen-1-one, and the CAS No. is 101205-02-1. Cycloxydim is a cyclohexene oxime herbicide that is used for the control of grass weeds of many agricultural and horticultural broad-leaved crops.

Cycloxydim was evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1992, when an acceptable daily intake (ADI) of 0–0.07 mg/kg body weight (bw) was established. Cycloxydim was reviewed by the present Meeting as part of the periodic re-evaluation programme of the Codex Committee on Pesticide Residues. New studies evaluated by the Meeting included studies with repeated percutaneous doses, studies of acute toxicity and genotoxicity with various metabolites, and 28-day and 90-day studies of toxicity in rats given repeated oral doses of metabolites.

Cycloxydim was used in the free acid form in most of the toxicological studies. However, because of chemical instability of the acid in animal feed and because of its low solubility in water, the sodium salt of cycloxydim was used in those studies that required water or feed as vehicle. The name "cycloxydim" refers to the acid form unless otherwise indicated. All the pivotal studies met the basic requirements of the relevant Organisation for Economic Co-operation and Development (OECD) guidelines, and certificates of compliance with good laboratory practice (GLP) and quality assurance (QA) were provided.

The chemical structure of cycloxydim is shown in Figure 1.

Figure 1. Chemical structure of cycloxydim



Evaluation for acceptable daily intake

1. Biochemical aspects

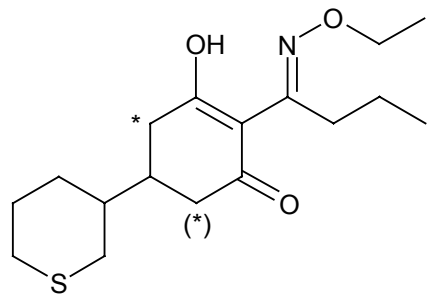
1.1 Absorption, distribution and excretion

Rats

To obtain information on the absorption, distribution and rate and route of excretion of cycloxydim, a study was performed in adult CD rats (body weight ~200 g; aged ~7 weeks [males] and 10 weeks [females]) using [^{14}C]cycloxydim labelled in the 4(6)-position of the cyclohexene ring (Figure 2). The radioactive test substance was sent by BASF to the contract research organization on three different occasions, all with the same batch number. The test substance was purified by the contract research organization ([cyclohexen-^{14}C]cycloxydim [BAS 517H]; batch No. 389211; specific radioactivity 398.9–462.5 MBq/mmol; radiochemical purity >94–100%).

The studies were conducted after oral administration of the free acid and its sodium salt and after intravenous administration of the sodium salt. The free acid was administered at a nominal dose of 10 mg/kg bw (no-effect level) or 300 mg/kg bw (toxic effect level). The sodium salt was administered at a nominal dose equivalent to 10 mg/kg bw as the free acid. In the studies in which excretion via the bile was determined, the animals were anaesthetized, and bile was collected via a catheter for up to 48 h after dosing. Urine and faeces were collected after 24 and 48 h.

Figure 2. Labelling position of the cyclohexene ring



* Denotes position of ^{14}C label

The experiments that were carried out are summarized in Tables 1, 2 and 3.

Table 1. Summary of excretion balance experiments

Parameter	Experimental dose group					
	E0	E1	E2	E3	E4	E5
Purpose	Excretion balance pretest	Excretion balance	Excretion balance	Excretion balance, 14 days unlabelled followed by single labelled dose	Excretion balance	Excretion balance
Route	Oral	Oral	Oral	Oral	Oral	Intravenous
Dose (mg/kg bw)	300	10	300	10	10	10
Test substance	Free acid	Free acid	Free acid	Free acid	Sodium salt	Sodium salt
No. of animals of each sex	2 males	5 males	5 males	5 males	5 males	5 males
	2 females	5 females	5 females	5 females	5 females	5 females
Duration[a] (h)	120	120	120	120	120	120
Samples	Urine, faeces, carcass, exhaled air	Urine, faeces, tissues	Urine, faeces, tissues	Urine, faeces, tissues	Urine, faeces, tissues	Urine, faeces, tissues

From Hawkins et al. (1986)

[a] Maximum time for collection of samples after dosing.

Table 2. Summary of biliary excretion experiments

Parameter	Experimental dose group		
	B1	B2	B3
Purpose	Biliary excretion	Biliary excretion	Biliary excretion
Route	Oral	Oral	Oral
Dose (mg/kg bw)	10	300	10
Test substance	Free acid	Free acid	Sodium salt
No. of animals of each sex	3 males	3 males	3 males
	3 females	3 females	3 females
Duration[a] (h)	48	48	48
Samples	Bile, urine, faeces	Bile, urine, faeces	Bile, urine, faeces

From Hawkins et al. (1986)

[a] Maximum time for collection of samples after dosing.

Table 3. Summary of plasma concentration experiments

Parameter	Experimental dose group					
	P1	P2	P3	P4	P5	P6
Purpose	Blood/plasma pharmaco-kinetics	Blood/plasma pharmaco-kinetics	Blood/plasma pharmaco-kinetics 7 daily doses	Blood/plasma pharmaco-kinetics 7 daily doses	Blood/plasma pharmaco-kinetics	Blood/plasma pharmaco-kinetics
Route	Oral	Oral	Oral	Oral	Oral	Intravenous
Dose (mg/kg bw)	10	300	10	300	10	10
Test substance	Free acid	Free acid	Free acid	Free acid	Sodium salt	Sodium salt
No. of animals of each sex	5 males 5 females	5 males 5 females	3 males 3 females	3 males 3 females	5 males 5 females	5 males 5 females
Duration[a] (h)	240	240	240	240	240	240
Samples	Blood	Blood	Blood, tissues	Blood	Blood	Blood

From Hawkins et al. (1986)

[a] Maximum time for collection of samples after dosing.

For the excretion balance experiments, the animals were placed in metabolism cages after dosing, and excreta were collected up to 120 h. In the single oral high-dose pretest, the exhaled air was collected in a carbon dioxide trapping system. At the end of the collection period, organs and tissues were prepared for analysis of residual radioactivity.

In the blood/plasma pharmacokinetics studies, blood was withdrawn at the following time points after dosing: 0.25, 0.5, 1, 2, 3, 5, 7, 12 (not group P1), 24, 48, 72, 96, 120, 168 and 240 h. Intravenously dosed rats were additionally bled at 5 min after dosing.

In the tissue distribution study, five male and five female rats were dosed with 10 mg/kg bw of the free acid for 7 days. Pairs of rats (one male and one female) were killed at 5, 12, 24, 72 and 120 h after the final dose. Radioactivity in 18 selected organs/tissues was determined. In addition, five males were treated for 7 days with the free acid and killed at 5, 12, 24, 72 and 120 h after the final dose. These animals were used to prepare whole-body autoradiographs. The excreta, organs and tissues obtained in this study were used for the identification of metabolites.

Urine samples collected during 24 h after the dose from male and female rats from group E2 (single dose 300 mg/kg bw, free acid) were pooled. The samples were adjusted to pH 10 and extracted with dichloromethane. The aqueous fraction was acidified (pH 3) and re-extracted with dichloromethane. Aliquots of the pH 10 and pH 3 extracts and the final aqueous fraction were subjected to thin-layer chromatography (TLC). An aliquot of the pH 3 extract was evaporated and redissolved in the respective high-performance liquid chromatography (HPLC) solvent system. Portions of this solution were subjected to semipreparative HPLC. A single major ultraviolet (UV)-active peak (metabolite I) was detected and further analysed using mass spectrometry. Similarly, aliquots of the pH 10 extract were analysed. Six separate peaks (metabolites II–VII) were collected and subjected to mass spectrometry. A sample of the aqueous fraction was subjected to TLC and HPLC analysis. Urine samples were also subjected to derivatization by means of a peroxidation and methylation reaction.

Bile from a male rat from group B2 (single dose of free acid at 300 mg/kg bw) was incubated overnight with β-glucuronidase. Following incubation, the sample was extracted with dichloromethane at pH 3 and pH 10, as described for urine. The extracts were analysed by TLC using solvent system A. UV detection indicated that the metabolites were present in the pH 10 fraction, and this was subjected to semipreparative HPLC using solvent system Y. Two major radioactive components were

Table 4. Excretion balance after oral administration of [cyclohexene-^{14}C]cycloxydim (free acid) to male and female rats

	Amount excreted (% of administered dose)					
	Dose (mg/kg bw)					
	10		300		10[a]	
	Male	Female	Male	Female	Male	Female
Urine						
- 0–8 h	39.86	28.44	24.06	15.09	30.49	24.85
- 8–24 h	38.94	42.21	52.66	51.61	38.63	39.60
- 24–48 h	3.80	4.87	5.77	13.27	4.06	4.53
- 48–120 h	1.42	2.03	2.35	3.44	2.87	3.14
Cage wash	0.40	0.37	0.63	0.61	0.52	0.70
Subtotal urine and cage wash	84.42	77.91	85.46	84.03	76.57	72.81
Faeces						
- 0–24 h	7.62	9.69	5.97	6.42	13.95	12.07
- 24–48 h	4.76	5.90	4.18	7.26	4.07	5.33
- 48–72 h	0.95	1.85	0.86	1.70	0.74	1.90
- 72–120 h	0.59	0.90	1.07	0.63	0.45	0.79
Subtotal faeces	13.92	18.33	12.06	16.02	19.21	20.10
Gut content	0.08	0.09	0.14	0.11	0.08	0.20
Tissues and carcass	0.31	0.45	0.40	0.59	0.37	0.75
Recovery	98.73	96.78	98.06	100.8	96.23	93.86

From Hawkins et al. (1986)

[a] 14 days of dosing with non-radiolabelled test substance followed by single oral ^{14}C-labelled dose.

designated metabolites VIII and IX. These metabolites were isolated by HPLC as described for urine and submitted to mass spectrometry. Samples of the two isolates were also analysed by TLC in solvent systems A and C, together with extracts of enzyme-treated bile and faeces and reference compounds cycloxydim and BH 517-T2S.

Faeces from male and female rats from group E2 (single dose of free acid at 300 mg/kg bw) and E3 (free acid at 10 mg/kg bw; 14 days non-labelled dose followed by single labelled dose) were used to analyse faecal metabolites. Extracts from male and female animals were analysed separately. For each experiment, faeces collected during 0–24 h and 24–48 h after dosing from each sex were pooled. Aliquots of pooled extracts were then processed, as described for urine, and chromatographed by means of TLC using solvent systems C and D.

Aliquots of liver and kidneys from a male and a female rat of dose group P3 (seven daily doses of free acid at 10 mg/kg bw) were used. Liver and kidney samples were homogenized with methanol. Liver samples were extracted a second time with methanol. Aliquots of the methanol solution were subjected to TLC using solvents A, B and C. Kidney samples were processed and chromatographed as described for the liver using solvent systems A and B. Co-chromatography with reference standards was used for the identification of metabolites.

The results of the excretion balance pretest demonstrated that no radioactivity was eliminated via the expired air. The excretion balance of cycloxydim is shown in Tables 4 and 5.

For the free acid, the results of the investigations indicate that the majority (78–85%) of the administered dose (single high or low) is eliminated via the urine. After a 14-day pretreatment, the

Table 5. Excretion balance after oral and intravenous administration of 10 mg/kg bw of [cyclohexene-^{14}C]cycloxydim (sodium salt) to male and female rats

	Amount excreted (% of administered dose)			
	Oral administration		Intravenous administration	
	Male	Female	Male	Female
Urine				
- 0–8 h	37.36	30.74	47.65	39.61
- 8–24 h	41.60	36.85	17.18	21.68
- 24–48 h	5.03	4.20	2.35	3.70
- 48–120 h	1.58	1.75	1.07	2.23
Cage wash	0.26	0.40	0.30	0.31
Subtotal urine and cage wash	85.83	73.94	68.55	67.53
Faeces				
- 0–24 h	11.76	18.61	12.30	12.18
- 24–48 h	4.59	4.42	3.95	7.76
- 48–72 h	0.90	2.12	1.17	1.94
- 72–120 h	0.42	0.61	0.45	0.72
Subtotal faeces	17.66	25.75	17.87	22.61
Gut content	0.08	0.09	0.06	0.09
Tissues and carcass	0.30	0.49	0.27	0.49
Recovery	103.9	100.3	86.76	90.70

From Hawkins et al. (1986)

amount eliminated via the urine is slightly lower (73–77%). For the cycloxydim sodium salt, the results indicate that the majority (74–86%) of the single oral dose of 10 mg/kg bw is eliminated via the urine, most being excreted within 24 h. A comparison of the excretion balance of the free acid and the sodium salt after oral administration (Table 6) indicates no biologically relevant difference.

The presence of radioactivity in the faeces after intravenous administration (Table 5) indicates that elimination via the bile plays a role. Moreover, the similarities in the excretion balance after oral and intravenous administration of 10 mg/kg bw indicate that both the free acid and the sodium salt of cycloxydim are well absorbed; the bioavailability of the oral dose was approximately 100%.

The results of the bile duct cannulation experiments confirm the notion that biliary excretion plays an important role in the elimination of the test substance. The fact that the amounts of radioactivity found in the faeces in the oral excretion balance studies are far lower than the biliary excretion (Table 6) demonstrates that part of the material excreted via the bile is reabsorbed (enterohepatic circulation) and subsequently eliminated renally.

The pharmacokinetic parameters of the free acid and the sodium salt of cycloxydim at a dose of 10 mg/kg bw were comparable, with the exception that the time to peak concentration in plasma (T_{max}) of the free acid was reached 5 h after oral administration, with peak concentrations (C_{max}) of 5 µg/ml and 6.8 µg/ml for males and females, respectively. In the case of oral administration of the sodium salt, T_{max} was 0.5–1 h, with C_{max} values of 8.4 µg/ml and 9.3 µg/ml for male and female rats, respectively. The C_{max} was similar after single or seven consecutive repeated oral doses of the corresponding concentration of the free acid. The area under the curve (AUC) obtained after the last of seven daily low doses was approximately twice as high as the AUC after a single oral dose, indicating very little accumulation over time at a dose of 10 mg/kg bw. Comparing the AUC obtained after a single low dose with that obtained after a single high dose demonstrates an approximate 60-fold

Table 6. Excretion via the bile after oral administration of [cyclohexene-^{14}C]cycloxydim—free acid and sodium salt—to male and female rats

	Amount excreted (% of administered dose)					
	Free acid				Sodium salt	
	Dose (mg/kg bw)					
	10		300		10	
	Male	Female	Male	Female	Male	Female
Bile						
- 0–24 h	59.89	64.24	50.67	46.28	51.30	63.67
- 24–48 h	1.38	0.98	5.55	8.45	3.5	2.38
Urine						
- 0–24 h	34.28	30.55	31.90	21.03	32.04	32.70
- 0–48 h	36.65	32.09	38.91	23.16	37.24	35.60
Subtotal urine and cage wash (0–48 h)	36.96	32.15	39.20	23.65	37.61	3.78
Faeces						
- 0–24 h	1.99	1.98	0.84	0.95	1.02	1.18
- 24–48 h	2.76	2.42	1.92	1.12	2.06	1.65
Gut content	0.04	0.03	0.13	17.76	0.06	0.04
Tissues and carcass	0.84	0.52	1.36	2.89	0.79	0.56
Recovery	102.0	100.3	98.83	100.2	95.32	104.1

From Hawkins et al. (1986)

Table 7. Pharmacokinetic parameters after oral and intravenous administration of [cyclohexene-^{14}C]cycloxydim—free acid and sodium salt—to male and female rats

Parameter	Experimental dose group					
	P1	P2	P3	P4	P5	P6
Route	Oral	Oral	Oral	Oral	Oral	Intravenous
Dose (mg/kg bw)	10	300	10	300	10	10
No. of doses	Single dose	Single dose	7 daily doses	7 daily doses	Single dose	Single dose
Test substance	Free acid	Free acid	Free acid	Free acid	Sodium salt	Sodium salt
C_{max} (µg/ml)	5.03 (M)	276 (M)	5.5 (M)	239 (M)	8.35 (M)	33.5 (M)
	6.82 (F)	297 (F)	6.8 (F)	263 (F)	9.26 (F)	39.5 (F)
T_{max} (h)	5 (M)	7 (M)	2 (M)	2 (M)	0.5 (M)	5 min (M)
	5 (F)	5 (F)	5 (F)	7 (F)	1.0 (F)	5 min (F)
AUC (µg·h/ml)	69.8 (M)	4731 (M)	130 (M)	6367 (M)	68.1 (M)	63.3 (M)
	111 (F)	5718 (F)	202 (F)	7203 (F)	108 (F)	97.0 (F)

From Hawkins et al. (1986)
F, female; M, male

increase. As the difference between the low and high dose levels was only 30-fold, these data indicate a more than proportional increase of the AUC with dose. The AUC after an intravenous administration of 10 mg/kg bw was nearly identical to the AUC after an oral dose of 10 mg/kg bw, indicating that all of the orally administered radioactivity was absorbed (Table 7).

Table 8. Tissue distribution and residual radioactivity in organs and tissues after seven daily oral treatments of rats with [cyclohexene-^{14}C]cycloxydim free acid at 10 mg/kg bw

Organ/tissue	Concentration (µg/g tissue)					
	Males			Females		
	5 h	24 h	72 h	5 h	24 h	72 h
Liver	8.86	1.19	0.51	15.3	3.17	1.51
Kidney	6.22	1.36	0.48	7.55	2.68	0.85
Thyroid	3.14	1.38	1.48	3.40	1.90	1.73
Intestinal tract	80.0	5.34	1.19	114	22.0	1.73
Adrenal gland	1.85	0.24	<0.15	2.96	0.75	0.28
Lungs	2.08	0.38	0.13	3.35	0.73	0.36
Plasma	4.79	0.70	0.18	7.57	1.44	0.54

From Hawkins et al. (1986)

The amounts of radioactivity found in the organs after seven daily treatments with 10 mg/kg bw in the tissue distribution study are shown in Table 8.

The results of the tissue distribution study indicated that after the last of seven daily doses, radioactivity in the organs rapidly declined over time. At 72 h after the last dose, radioactivity was less than 1 µg/g tissue for almost all organs. The highest amounts of radioactivity were found in the liver and the kidneys (i.e. those organs involved in the excretion of the test substance). Compared with the plasma level, concentrations in the other organs were equal or lower. There was no evidence for bioaccumulation of the test substance.

The results of the whole-body autoradiography confirmed the findings of the tissue distribution studies. Highest amounts of radioactivity were detected in the intestinal tract, liver, kidneys, blood and lungs. There were no organs with unusually high levels of radioactivity.

The study described in this report was conducted in compliance with GLP of the United States Environmental Protection Agency (USEPA), OECD and Japan. A QA statement was attached (Hawkins et al., 1986).

1.2 Biotransformation

The result of the above-described study was used for elucidation of metabolites. The urine samples, faecal extracts, bile and organs were analysed. The analysis indicated that several metabolites were formed. The majority of them could be isolated and identified spectrometrically. As far as possible, the proposed structures for the metabolites were corroborated by comparison with synthetic standards.

Patterns of metabolites in the urine were qualitatively and quantitatively similar after administration of 10 mg/kg bw of either the free acid (Table 9) or the sodium salt (Table 10). At a dose of 300 mg/kg bw (free acid), the qualitative distribution of urinary metabolites was again similar; quantitatively, however, the data indicated that the test substance was less extensively metabolized. The major metabolite in all cases was the sulfoxide of cycloxydim (BH 517-TSO). The next most important metabolite was BH 517-T1SO, resulting from *N*-de-ethoxylation of BH 517-TSO. Other less important metabolites in the urine were BH 517-T1SO$_2$ (sulfone of BH 517-T1SO), BH 517-T2SO (Beckmann rearranged product of the parent sulfoxide) and unchanged parent (cycloxydim). The presence of minor metabolites hydroxylated at the 5-position of the cyclohexene ring of the parent was indicated after oxidation and methylation of a urine sample. Patterns of metabolites in the bile were similar after the administration of either the free acid or the sodium salt of cycloxydim

Table 9. Summary of identified metabolites in urine after single low, single high and multiple low dose administration of [^{14}C]cycloxydim free acid

Metabolite identity	Radioactivity (% of administered dose)					
	Dose group E1 (10 mg/kg bw single oral)		Dose group E2 (300 mg/kg bw single oral)		Dose group E3[a] (10 mg/kg bw multiple oral)	
	Males	Females	Males	Females	Males	Females
Polars	7.5	10.1	3.5	3.4	3.4	8.2
Unknown	1.6	1.9	0.7	0.7	0.7	2.3
A	3.9	4.1	2.4	1.6	2.1	4.7
B	6.6	8.4	3.1	2.9	2.5	B & C combined: 9.3
C	5.1	3.8	2.9	2.5	1.5	
BH 517-T1SO	12.9	9.9	6.5	5.5	5.2	7.7
D	3.1	2.2	4.5	3.8	2.9	2.4
BH 517-T2SO	7.4	5.5	5.1	4.5	3.6	3.8
BH 517-TSO, BH 517-T1SO$_2$	29.9	23.6	46.7	40.5	43.8	24.1
BH 517-TSO$_2$				0.4	1.2	
Cycloxydim	Combined: 0.8	Combined: 1.1	Combined: 1.3	0.5	0.7	Combined: 2.0
Unknown				0.2	0.1	

From Hawkins et al. (1986)

[a] Fourteen non-radiolabelled doses followed by a single ^{14}C-labelled dose.

Table 10. Summary of identified metabolites in urine after single oral and intravenous low dose administration of [^{14}C]cycloxydim sodium salt

Metabolite identity	Radioactivity (% of administered dose)			
	Dose group E4 (10 mg/kg bw single oral)		Dose group E5 (10 mg/kg bw single intravenous)	
	Males	Females	Males	Females
Polars	6.5	10.0	5.7	11.2
Unknown	1.6	3.0	1.2	2.8
A	3.9	4.9	3.9	4.2
B	5.4	5.9	4.5	5.7
C	5.6	4.3	3.7	3.3
BH 517-T1SO	10.5	9.8	8.4	8.1
D	3.6	2.1	2.4	1.6
BH 517-T2SO	6.9	4.5	4.5	5.2
BH 517-TSO, BH 517-T1SO$_2$	33.7	21.4	28.9	18.4
BH 517-TSO$_2$	0.7		0.5	
Cycloxydim	0.1	Combined: 1.6	0.3	Combined: 0.9
Unknown	0.5		0.4	

From Hawkins et al. (1986)

Table 11. Summary of identified metabolites in bile after single oral dose administration of [^{14}C]cycloxydim free acid or sodium salt

Metabolite identity	Radioactivity (% of administered dose)							
	Dose group B (10 mg/kg bw single oral – free acid)				Dose group B (10 mg/kg bw single oral – sodium salt)			
	Males		Females		Males		Females	
	Non-enzyme treated	Enzyme treated	Non-enzyme treated	Enzyme treated	Non-enzyme treated	Enzyme treated	Non-enzyme treated	Enzyme treated
Polars	47.8	5.1	57.4	8.2	51.1	8.5	60.9	10.7
Unknown	1.3	0.7	2.9	0.8	1.3	0.4	1.6	0.5
A	1.7	1.3	2.0	1.1	1.8	1.0	2.3	1.1
B	2.0	0.9	2.2	1.6	1.9	0.5	2.1	1.1
C	3.1	1.3	1.2	1.1	2.2	1.4	2.1	0.9
BH 517-T1SO	1.7	1.0	1.3	1.4	1.4	0.9	1.8	0.7
D	10.6	5.7	7.6	3.8	9.3	6.6	6.1	1.9
BH 517-TSO, BH 517-T1SO$_2$	Combined: 5.4	Combined: 6.9	Combined: 3.5	Combined: 11.5	Combined: 14.5	Combined: 34.3	Combined: 7.5	Combined: 22.9
BH 517-T2SO + E	Combined: 20.3	Combined: 50.2	Combined: 14.2	Combined: 39.2	7.0	13.7	4.6	11.4
E	—	—	—	—	2.3	2.9	3.0	1.0
Unknown	2.7	1.9	4.1	2.3	2.1	—	3.2	1.4
BH 517-T2S	2.7	7.7	3.0	9.3	3.0	10.8	4.0	18.2
Cycloxydim	0.6	17.3	0.8	19.6	2.0	18.8	0.8	28.1
Unknown	0.1	0.1	0.1	0.1	—	0.1	—	0.1

From Hawkins et al. (1986)

(Table 11). Following enzymatic hydrolysis, the major metabolites in the bile corresponded to BH 517-TSO (including BH 517-T1SO$_2$), unchanged parent and BH 517-T2S (*N*-de-ethoxylated and rearranged product of the parent).

Methanol extracts of the liver contained two major components, which corresponded to parent and BH 517-TSO. The pattern of metabolites in the kidneys was similar to that in the urine (Table 12).

The pattern of metabolites in the urine was similar for the free acid and the sodium salt of cycloxydim, and AUC data indicated that elimination was saturable at higher doses. The major metabolite in the urine and bile was the sulfoxide of cycloxydim, BH 517-TSO. Additional metabolites identified were BH 517-T1SO (derived from *N*-de-ethoxylation of BH 517-TSO), BH 517-T1SO$_2$ and BH 517-T2SO. Only small amounts of unchanged parent compound were detected in the urine.

In the faeces, a total of nine different radioactive fractions were detected in the single oral high and low dose groups (free acid). Unchanged parent accounted for 0.7% (males) to 0.9% (females) of the dose in the high dose group and 0.8% (males) to 0.5% (females) in the low dose group. The values for polars were 1.6% (males) to 2.2% (females) (300 mg/kg bw) and 2.2% (males) to 3.2% (females) (10 mg/kg bw). A further component designated "F" accounted for 1.4% (males) to 2.3% (females) (300 mg/kg bw) and 3.3% (males) to 3.7% (females) (10 mg/kg bw). The other components were not designated or identified, but each accounted for less than 1% of the dose.

The metabolic pathway of cycloxydim is shown in Figure 3.

Table 12. Summary of identified metabolites in liver and kidney after seven daily oral low dose administrations of [^{14}C]cycloxydim free acid to rats[a]

Metabolite identity	% of radioactivity in specified organ		
	Liver: 10 mg/kg bw – seven daily doses		Kidney: 10 mg/kg bw – seven daily doses
	Males	Females	Pooled males/females
Polars	7.3	6.4	8.2
Unknown (roi2)	3.6	5.8	3.6
B, C	—	—	7.2
BH 517-T1SO	3.6	5.1	6.1
D	6.0	5.2	5.3
BH 517-TSO	30.2	26.6	—
BH 517-T2SO	—	—	8.8
BH 517-TSO, BH 517-T1SO$_2$	—	—	44.3
Unknown (roi 6)	2.3	1.2	—
G	7.9	5.6	—
Unknown (roi 8)	2.7	1.6	4.0
Cycloxydim	18.6	16.0	3.4
Unknown (roi 10)	<0.01	0.9	0.2

From Hawkins et al. (1986)
roi, region of interest
[a] Liver and kidney components were resolved with TLC solvent A.

The study described in this report was conducted in compliance with GLP of the USEPA, OECD and Japan. A QA statement was attached (Hawkins et al., 1986).

2. Toxicological studies

2.1 Acute toxicity

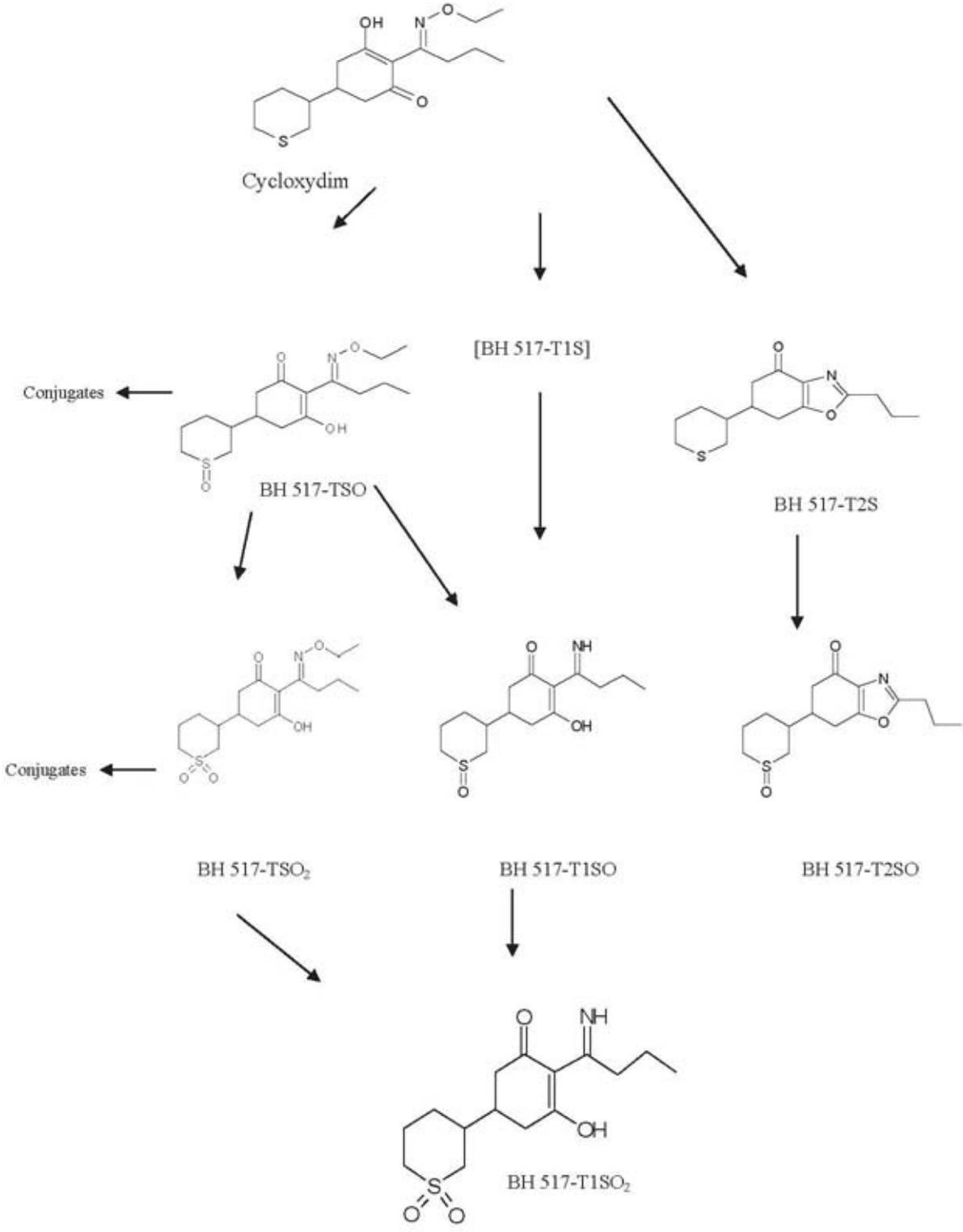
The oral median lethal dose (LD_{50}) of cycloxydim was 3940 mg/kg bw in rats and >5000 mg/kg bw in mice. No specific clinical signs were observed. Macroscopic findings in rats that died after receiving high oral doses by gavage indicated irritation of the gastric mucosa. The dermal LD_{50} in rats was >2000 mg/kg bw, a dose of 2000 mg/kg bw causing neither mortality nor systemic toxicity. No local skin reaction was observed at the application site. When cycloxydim was administered by inhalation, the median lethal concentration (LC_{50}) was >5.28 mg/l of air (4 h exposure). Cycloxydim was not an irritant in a study of skin and eye irritation in rabbits or a skin sensitizer in the guinea-pig maximization test.

The results of the studies on acute toxicity of cycloxydim are presented in Table 13. All the studies were conducted as per OECD 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 using cycloxydim sodium salt or cycloxydim free acid. Cycloxydim sodium salt as test substance was applied instead of

Figure 3. The metabolic pathway of cycloxydim



Source: Hawkins et al. (1986)

Table 13. Summary of acute toxicity studies with cycloxydim

Species	Strain	Sex	Route	Batch No.; purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Results	Reference
Mouse	NMRI/ MAN/ WIGA	M + F	Oral	N 64; 94.8	M: >5000 F: >5000 M + F: >5000	—	[a]	Kieczka & Kirsch (1985b)
Rat	Wistar	M + F	Oral	N 11; 92.2	M: 4420 F: 3830 M + F: 3940	—	[b]	Kieczka & Kirsch (1984a)
Rat	Wistar	M + F	Dermal	N 11; 92.2	>2000	—	[c]	Kieczka & Kirsch (1984b)
Rat	Wistar	M + F	Inhalation 4 h (head–nose exposure)	N 64; 94.8	—	>5.28 (liquid aerosol; MMAD 50% 1.6 µm)[d]	[e]	Klimisch et al. (1985)
Rabbit	White Vienna	M + F	Acute dermal irritation/ corrosivity	N 11; 92.2	—	—	Non-irritant	Kieczka & Kirsch (1984c)
Rabbit	White Vienna	M + F	Eye irritation	N 11; 92.2	—	—	Non-irritant	Kieczka & Kirsch (1984d)
Guinea-pig	Pirbright white, Dunkin Hartley HOE DHPK SPF-LAC	F	Skin sensitization effects (guinea-pig maximization)	N 64; 94.8	—	—	Non-sensitizer	Kieczka & Kirsch (1985a)

F, female; M, male; MMAD, mass median aerodynamic diameter

[a] At the highest dose level, mortality was restricted to one male mouse, which died within 1 day after test substance administration. Clinical signs recorded were dyspnoea, apathia, abnormal position, staggering, paresis, absent pain reflex, narcotic-like state, twitching, piloerection, imbalance and poor general state. The onset of signs was observed between 30 min and 1 day after dosing. These signs were observed in animals that survived up to 1 day of the study and were dose dependent starting at 1470 mg/kg bw. At study termination, the surviving animals were free of signs. They can be considered to be unspecific, with no substantial difference between sexes. Macroscopic examination in the mouse that died revealed a yellow-brown discoloration of the liver. No substance-related findings were noted in animals that survived.

[b] Clinical signs recorded were dyspnoea, apathia, abnormal position, staggering, atonia, paresis, tremors, twitching, spastic gait, piloerection, exsiccosis, salivation, lacrimation and poor general state. The onset of signs was observed between 30 min and 5 h after dosing. Signs were observed in animals that survived up to day 7 of the study. They were dose dependent starting at 2150 mg/kg bw. No signs were noted at the lowest dose level of 1210 mg/kg bw. At study termination, the surviving animals were free of signs. The signs can be considered to be unspecific, with no substantial difference between sexes. Macroscopic examination in animals that died revealed bloody ulceration of the glandular stomach and haematinized contents of the intestine in several cases. No substance-related findings were noted in animals that survived.

[c] There were no signs of systemic intoxication, mortalities or local signs of irritation. No macroscopic findings were noted at sacrifice.

[d] MMAD calculated as acid.

[e] There were no mortalities in the study. During exposure to cycloxydim, weak attempts to escape were noted, as well as eyelid closure and shallow and slightly jerky respiration. Later during exposure, respiration was jerky. After exposure, slight piloerection and slightly jerky respiration were noted. No abnormalities apart from slight alopecia on the head were noted on day 6 after exposure. The body weight gain in males and females revealed no substance-related effects. No macroscopic findings were noted at study termination (day 14).

cycloxydim free acid if the free acid could not be used in the feed because of its insufficient stability and could also not be administered in the drinking-water on account of its low solubility.

In rodent short-term toxicity studies, cycloxydim sodium salt was administered via the drinking-water after it was found that the sodium salt was not sufficiently stable when mixed in rodent feed. After only 1 day, the active ingredient concentration in the mixture of feed and test substance was only about 78% of the initial value, and after 8 days, only about 50% was detected.

In toxicity studies in dogs, however, dietary administration of cycloxydim sodium salt was possible because fresh test substance feed mixture could be prepared each day shortly before feeding. In addition, sufficient stability could be verified analytically for the short period until the feed had been completely consumed.

In all studies, the feed concentration values (in parts per million [ppm]) and test substance intakes (in mg/kg bw per day) refer to cycloxydim as the acid rather than as its sodium salt. Continuous test substance administration in these studies was verified by regular determination of the consumption of drinking-water containing the test substance (2 times per week).

The results of these studies are characterized by clinicochemical changes associated with changes in water and food consumption and effects on the liver. Effects on erythrocytes were seen only in dogs at high doses. Where the test substance was administered in the drinking-water, the reduction in water consumption is regarded to be a palatability effect rather than a specific adverse effect.

(a) Oral route

Mice

In a 4-week range-finding study designed to determine appropriate dose levels for a long-term carcinogenicity study, 10 B6C3F1/Cr IBR mice of each sex per dose were administered cycloxydim as the sodium salt (purity 94.8%; batch No. N 64) via the drinking-water at concentrations of 0, 300, 1000, 3000 and 9000 ppm (equal to 0, 59, 189, 462 and 1008 mg/kg bw per day for males and 0, 63, 218, 591 and 1177 mg/kg bw per day for females, respectively). The test substance intake over the entire study was calculated as the free acid for the purpose of this assessment based on the weekly test substance intake data provided in the report. The mice were 43 days old at the beginning of the administration of the test substance, and the mean body weights were 19.5 g and 18 g for males and females, respectively.

Food consumption and body weight were determined once per week. Water consumption was determined twice per week. The health status of the animals was checked daily. At the end of the test substance administration, blood samples were taken for clinicochemical examinations. All animals were sacrificed after 4 weeks. The organ weights of liver, kidneys, heart, brain, spleen, thymus, testes and adrenals were determined; selected organs were assessed by gross pathology. Subsequently, a histopathological examination of the liver, kidney, stomach, jejunum and lung and of all gross lesions was carried out.

There were no test substance–related mortalities or clinical signs of toxicity in any treatment group. Body weight gain was reduced in the 9000 ppm group. A temporary reduction in body weight was observed in most of the animals of all test groups on day 21 only. As the controls also lost some weight at this time, this isolated change in body weight development is not considered to be test substance related. Food consumption of animals of the 9000 ppm groups was reduced. Water consumption was reduced in males and females of the 9000, 3000 and 1000 ppm groups, as well as in females of the 300 ppm group. The extent of the reduction varied over time; in general, the water consumption reduction was approximately 50% for the 9000 ppm animals, varied between 5% and 35% for the 3000 ppm animals, ranged between 3% and 10% for the 1000 ppm animals and was 13% in the 300 ppm females.

There were no test substance–related haematological changes in any of the treatment groups. Clinicochemical examinations revealed an increase in urea values in 9000 ppm males and females. In addition, a decrease in cholesterol level was seen in 9000 and 3000 ppm animals (Table 14). Owing to the small amount of blood available, it was not possible to evaluate all clinicochemical parameters in all animals. Therefore, for many clinicochemical parameters, the number of available data points per sex and dose varied (occasionally as low as 2 out of 10); hence, the results of the clinicochemical investigation should be viewed with caution.

Table 14. Clinicochemical parameters measured in a range-finding study in mice administered cycloxydim as the sodium salt for 4 weeks

Parameter	Concentration in drinking-water (ppm)				
	0	300	1000	3000	9000
Urea (mmol/l)					
- Males (N[a] = 4–8)	5.40	5.16	6.27	6.34	9.55
- Females (N = 3–7)	4.25	10.12	5.52	5.98	11.39
Cholesterol (mmol/l)					
- Males (N = 2–5)	1.78	1.63	1.47	0.85	0.80
- Females (N = 2–4)	1.97	1.23	1.68	0.45	0.53

From Kuehborth et al. (1986a)
[a] N = number of blood samples evaluated for assessment of parameter.

Table 15. Organ weight determinations in a range-finding study in mice administered cycloxydim as the sodium salt for 4 weeks

Parameter	Concentration in drinking-water (ppm)									
	0		300		1000		3000		9000	
Heart weight – males										
- Absolute, g (% of controls)	0.110	(—)	0.107	(97)	0.106	(96)	0.106	(96)	0.092**	(84)
- Relative, % (% of controls)	0.562	(—)	0.561	(100)	0.560	(100)	0.551	(98)	0.533*	(95)
Liver weight – males										
- Absolute, g (% of controls)	0.768	(—)	0.796	(104)	0.810	(105)	0.862	(112)	0.780	(102)
- Relative, % (% of controls)	3.918	(—)	4.176*	(107)	4.264*	(109)	4.491**	(115)	4.512**	(115)
Thymus weight – females										
- Absolute, g (% of controls)	0.0523	(—)	0.0610	(117)	0.0549	(105)	0.0576	(110)	0.0637*	(122)
- Relative, % (% of controls)	0.2889	(—)	0.3506	(121)	0.3006	(104)	0.3352	(116)	0.3943**	(136)

From Kuehborth et al. (1986a)
* $P < 0.05$; ** $P < 0.01$ (Williams t-test)

Organ weight determinations revealed a test substance–related decrease in absolute weights of the kidneys, spleen, testes and brain in 9000 ppm male mice and of the heart, kidney and liver in 9000 ppm females. Because there were no similar changes in relative organ weights, these reductions were considered to be related to the reduction in body weight found at this dose level. Both absolute and relative heart weights were decreased in 9000 ppm male mice only. Relative liver weights were slightly increased in male mice of all dose groups. There was, however, no statistically significant increase in absolute liver weight in males at any dose level. In the high dose group females, the absolute and relative thymus weights were significantly increased (Table 15).

Gross necropsy did not show any test substance–related changes. The histopathological investigations revealed hydropic vacuolar degeneration in hepatocytes of two male 9000 ppm mice. There were no other test substance–related changes observed during the histopathological evaluations.

In conclusion, the administration of cycloxydim sodium salt to mice via drinking-water over a period of 28 days elicited toxic effects in all dose groups. The target organs appear to be the liver and kidney, as can be seen from degeneration of hepatocytes in two males of the high dose group, the increase in relative liver weights in male mice of all dose groups and the increase in urea values in mice of both sexes of the highest dose group. Based on significantly increased relative liver weights

(over 115%) at 3000 ppm and 9000 ppm in combination with altered clinicochemical parameters (enhanced plasma urea level and decreased plasma cholesterol) and the occurrence of hydropic vacuolar parenchymal degeneration of hepatocytes in two animals in the highest dose group, a (limited) NOAEL could be set as 1000 ppm (equal to 189 mg/kg bw per day in male mice and 218 mg/kg bw per day in female mice). A QA statement was attached. The study was conducted as per OECD test guideline 407, with the following deviation: clinicochemical investigations were limited to total bilirubin, urea, potassium chloride, triglycerides, cholesterol and alkaline phosphatase; haematological parameters were not investigated; and histopathological examination was limited to liver, kidneys, stomach, jejunum and lungs (Kuehborth et al., 1986a).

In a second 4-week range-finding study, 10 B6C3F1 mice of each sex per group were administered cycloxydim as the sodium salt (purity 94.8%; batch No. N 64) via the drinking-water at concentrations of 0, 30, 100, 300 and 900 ppm (equal to 0, 7.3, 22.5, 68.3 and 204 mg/kg bw per day for males and 0, 8.8, 28.3, 82.3 and 242 mg/kg bw per day for females, respectively). The test substance intake over the entire study was calculated based on the weekly test substance intake data provided in the report. The mice were 42 days old at the beginning of the administration of the test substance, and the mean weights were 19.1 g and 15.7 g for males and females, respectively.

Food consumption and body weight were determined once a week. Water consumption was determined twice per week. The health of the animals was checked each day. At the end of the administration period, blood samples were taken for a limited clinicochemical examination (activity of lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase). All animals were sacrificed after 4 weeks. The liver weights were determined and assessed by gross pathology. As there were no histopathological changes in the previous mouse study (Kuehborth et al., 1986a) at doses up to 1000 ppm, histopathological examinations were not carried out in this study.

There were no test substance–related mortalities or clinical signs of toxicity in any treatment group. There were no effects on body weight or food consumption. Water consumption was reduced in males and females of the 900 ppm group as well as in females of the 300 ppm group. The extent of the reduction varied over time; in general, the water consumption reduction was approximately 4–9% for the 900 ppm females and 4–7% for the 300 ppm females. In the 900 ppm males, water consumption was reduced by approximately 8% during the first week of the study only. As already discussed above for the previous mouse study (Kuehborth et al., 1986a), because the test substance was administered via the drinking-water, the reduction of water consumption is to be regarded as a palatability effect rather than a specific adverse effect.

There were no test substance–related clinicochemical changes in any of the treatment groups. Organ weight determinations revealed an increase in absolute and relative liver weights in male mice of the 900, 300 and 100 ppm groups. Relative liver weights were increased in 900 ppm females (Table 16).

Gross necropsy did not show any test substance–related changes.

In the previous mouse study (Kuehborth et al., 1986a), there were no histopathological changes at dose levels up to 1000 ppm. From these data, it is inferred that the marginally increased liver weights observed in this study were also without histopathological changes and thus considered to represent adaptive rather than adverse effects. The NOAEL was established at the highest dose level of 900 ppm, because the marginally increased liver weights without histopathological changes were considered to represent adaptive rather than adverse effects. A QA statement was attached. The study was conducted as per OECD test guideline 407, with the following deviations: no histopathology was conducted; and weight determinations were undertaken from limited organs only (Kuehborth et al., 1986b).

With the few available parameters in both studies, an overall NOAEL was set at 1000 ppm (equal to 189 mg/kg bw per day in male mice and 218 mg/kg bw per day in female mice), on the

Table 16. Absolute and relative liver weights in a 4-week range-finding study in mice administered cycloxydim as the sodium salt in the drinking-water

Parameter	Concentration in drinking-water (ppm)									
	0		30		100		300		900	
Liver weight – males										
- Absolute, g (% of control)	0.821	(—)	0.871	(106)	0.910**	(111)	0.887**	(108)	0.923**	(112)
- Relative, % (% of control)	4.019	(—)	4.103	(102)	4.162*	(104)	4.182*	(104)	4.362**	(109)
Liver weight – females										
- Absolute, g (% of control)	0.818	(—)	0.782	(96)	0.773	(94)	0.788	(96)	0.845	(103)
- Relative, % (% of control)	4.461	(—)	4.356	(98)	4.378	(98)	4.438	(99)	4.716**	(106)

From Kuehborth et al. (1986b)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

basis of a significant increase in relative liver weights at concentrations of 3000 ppm and 9000 ppm in combination with altered clinicochemical parameters and the occurrence of hydropic vacuolar parenchymal degeneration of hepatocytes in the first study.

Rats

In a range-finding study, cycloxydim as the sodium salt (purity 92.8%; batch No. N 11) was administered to groups of five male and five female Wistar rats via their drinking-water at concentrations of 0, 300, 1000, 3000 and 9000 ppm (equal to 0, 32, 102, 272 and 683 mg/kg bw per day for males and 0, 35.3, 106, 252 and 678 mg/kg bw per day for females, respectively) for 4 weeks. The rats were 42 days old at the beginning of the administration of the test substance, and the mean weights were 162.56 g and 150.28 g for males and females, respectively.

Food consumption and body weight were determined once a week. Drinking-water consumption was measured twice per week. The health status of the animals was checked daily. Blood samples from five animals of each sex per dose group for clinicochemical and haematological examinations were taken at 12 and 25 days after the beginning of administration. Urinalysis was performed after 22 days of test substance administration. At the end of the 4-week administration period, all animals were fasted for 16 h and then sacrificed by decapitation after they had been anaesthetized with carbon dioxide. Organ weights were determined, and gross pathological as well as histopathological examinations were carried out.

One female of the 9000 ppm group died on day 12 of the study. There were no further mortalities in any of the treatment groups. The general health condition of all males and females of the 9000 ppm group appeared to be temporarily impaired; in two high dose group males, a slight red incrustation of the nose was noted; a further male showed slight redness of the nose. On day 8, ruffled fur was observed in one female, which died 4 days later. None of the symptoms mentioned were observable at the end of the study period.

Body weight gain was reduced in males and females of the 9000 ppm group as well as in 3000 ppm group females (Table 17).

Food consumption of animals of the 9000 ppm group as well as 3000 ppm females was reduced throughout the entire study period. In the males of the 3000 ppm group, a temporary reduction of food consumption occurred during the first week only (Table 18).

Water consumption was reduced in males and females of all dose groups. While the extent of this reduction varied during the 4-week administration period, a dose-related effect on water consumption was obvious.

There were no test substance–related haematological changes in any of the treatment groups. Clinicochemical examinations performed at the end of the study revealed an increase in urea values

Table 17. Body weight at day 28 and body weight gain in a range-finding study in rats administered cycloxydim as the sodium salt in drinking-water

Concentration in drinking-water (ppm)	Males				Females			
	Body weight (day 28)		Body weight gain (days 1–28)		Body weight (day 28)		Body weight gain (days 1–28)	
	g	% of control	g	% of control	g	% of control	g	% of control
0	302.40	—	136.60	—	217.60	—	66.40	—
300	310.00	103	146.60	107	215.60	99	65.80	99
1000	296.80	98	137.20	100	216.20	99	64.20	97
3000	294.20	97	130.20	95	198.20	91	50.40	76
9000	216.60	72	56.60**	41	173.75	80	23.50**	35

From Kuehborth et al. (1986c)
** $P < 0.01$ (Williams *t*-test)

Table 18. Mean food intake during 4-week treatment of rats with cycloxydim as the sodium salt in drinking-water

Concentration in drinking-water (ppm)	Males				Females			
	Days 1–28		Days 1–7		Days 1–28		Days 1–7	
	g/day	% of control	g/day	% of control	g/day	% of control	g/day	% of control
0	26.07	—	25.49	—	20.22	—	19.57	—
300	26.75	103	25.57	100	19.65	97	19.80	101
1000	25.53	98	24.20	95	19.57	97	19.57	100
3000	25.44	98	23.40	92	17.50	87	16.66	85
9000	17.91	69	13.51	53	14.80	73	9.83	50

From Kuehborth et al. (1986c)

and slightly increased chloride and sodium levels at 9000 ppm in both sexes. At 3000 ppm, a marginal increase in urea concentration was observed in females after 12 days on study, but not after 25 days; this finding was therefore not considered related to treatment. In addition, triglyceride levels were reduced at 9000 ppm in both sexes. A slight increase in cholesterol level in 9000 ppm males was observed on day 12, but not on day 25; therefore, this finding is not considered to be test substance related. A slight decrease in alkaline phosphatase activity observed in high dose group females was not considered to be of toxicological significance (Table 19).

Organ weight determinations revealed a test substance–related decrease in absolute weights of the heart, liver, kidneys, spleen, adrenals and brain in 9000 ppm male rats and of heart and adrenal in 9000 ppm females. Because there was no similar decrease in relative weights, these reductions were considered to be related to the reduction in body weight at this dose level. Similarly, statistically significantly increased relative liver and kidney weights seen at 9000 ppm in males and at 3000 ppm in females were considered to be a consequence of reduced body weights. In females, increases in relative liver and kidney weights at 9000 ppm were judged to be a direct substance-related effect on the organs, because the corresponding absolute organ weights were not decreased and the relative weight increase was higher than the respective decrease in mean terminal body weight (Table 20).

Gross necropsy and histopathological investigations did not show any test substance–related changes.

Table 19. Clinicochemical parameters at day 25 in rats administered cycloxydim as the sodium salt in drinking-water

Parameter	Concentration in drinking-water (ppm)				
	0	300	1000	3000	9000
Urea (mmol/l)					
- Males	6.98	7.06	6.90	7.24	8.19**
- Females	6.97	7.25	7.61	7.76	9.23**
Sodium (mmol/l)					
- Males	141.91	141.24	139.08*	140.71	143.93
- Females	141.25	139.97	141.29	143.81	145.21*
Chloride (mmol/l)					
- Males	98.10	98.15	96.28*	98.88	100.55
- Females	99.93	98.60	98.87	100.54	102.11
Triglycerides (mmol/l)					
- Males	2.16	2.29	1.83	2.50	1.13*
- Females	1.45	1.35	1.63	1.17	1.00
Cholesterol (mmol/l)					
- Males	1.55	1.77	1.72	1.90	1.68
- Females	1.56	1.32	1.66	1.41	1.42
Alkaline phosphatase (µkat/l)					
- Males	10.69	11.71	9.93	10.99	9.74
- Females	7.56	7.32	7.26	7.68	5.34**

From Kuehborth et al. (1986c)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

In view of the above, it can be concluded that the dose level of 9000 ppm resulted in temporary poor general state and a clear reduction in food consumption and body weight development with associated organ weight decreases. Slight reductions in body weight and food intake were also observed in females at 3000 ppm. There was a reduction in water consumption at all dose levels. As the test substance was administered via the drinking-water, the reduction of water consumption is regarded to be a palatability effect rather than a specific adverse effect. At 9000 ppm, clinicochemical changes that were considered treatment related comprised increased urea, sodium and chloride levels in both sexes (all considered secondary to reduced water intake), reduced triglycerides in both sexes and decreased alkaline phosphatase in females. With the exception of increased relative liver and kidney weights in high dose group females, which were considered to represent a direct organ-specific effect of cycloxydim, the other changes in relative organ weights were secondary to reduced body weights. In the absence of any associated clinicochemical or histopathological changes, the increased relative liver and kidney weights in females at 9000 ppm are considered to represent adaptive rather than adverse effects.

The NOAEL was assessed to be 3000 ppm in males (equal to 272 mg/kg bw per day) and 1000 ppm in females (equal to 106 mg/kg bw per day), based on reductions in food consumption and body weight gain at the next higher dose level. Neither the GLP certification nor any QA statement was attached. However, the study was conducted as per OECD test guideline 407 (adopted in 1981), with the following deviations: no functional observational battery (FOB) (not required in the 1981 guideline); no gross examination of large intestines, bone marrow, lymph node, peripheral nerve or spinal cord; and no histopathology of large intestines, spinal cord, bone marrow, heart, trachea, lung,

Table 20. Organ weights in rats administered cycloxydim as the sodium salt in drinking-water

Parameter	Concentration in drinking-water (ppm)									
	0		300		1000		3000		9000	
Terminal body weight, g (% of control)										
- Males	271.66	(—)	281.16	(103)	267.7	(99)	267.46	(98)	200.72**	(74)
- Females	196.52	(—)	193.8	(99)	194.02	(99)	180.76	(92)	162.68**	(83)
Absolute liver weight, g (% of control)										
- Males	9.326	(—)	9.848	(106)	9.296	(100)	9.568	(103)	7.854*	(84)
- Females	6.73	(—)	7.042	(105)	7.12	(106)	6.784	(101)	7.085	(105)
Relative liver weight, % (% of control)										
- Males	3.427	(—)	3.501	(102)	3.471	(101)	3.574	(104)	3.911**	(114)
- Females	3.419	(—)	3.631	(106)	3.667	(107)	3.766*	(110)	4.347**	(127)
Absolute kidney weight, g (% of control)										
- Males	2.162	(—)	2.312	(107)	2.054	(95)	2.310	(107)	1.820**	(84)
- Females	1.554	(—)	1.578	(102)	1.67	(107)	1.596	(103)	1.623	(104)
Relative kidney weight, % (% of control)										
- Males	0.795	(—)	0.823	(104)	0.768	(97)	0.865*	(109)	0.907**	(114)
- Females	0.793	(—)	0.815	(103)	0.862	(109)	0.892	(112)	1.003**	(126)

From Kuehborth et al. (1986c)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

thymus, urinary bladder, prostate/uterus, pituitary gland, brain, skeletal muscle, ovaries, lymph nodes or peripheral nerve (Kuehborth et al., 1986c).

In a 90-day study, cycloxydim (purity 94.8%; batch No. N 64) was administered as the sodium salt to groups of 10 male and 10 female Wistar rats via their drinking-water at dose levels of 0, 30, 100, 300, 900 and 2700 ppm (equal to 0, 2.2, 7.3, 22, 72 and 178 mg/kg bw per day for males and 0, 3.2, 10, 28, 74 and 201 mg/kg bw per day for females, respectively). In order to study the possible reversibility of effects, additional groups, each consisting of 10 males and 10 females, were administered dose levels of 0, 900 and 2700 ppm, respectively, for 3 months and subsequently maintained on test substance–free drinking-water for an additional 6 weeks as recovery groups. At the initiation of the study, the age of the animals was 42 days, and average weights were 192.9 g and 134.7 g for males and females, respectively.

The animals were observed daily for clinical signs and mortalities. A thorough examination, including palpation, was performed once per week. Body weight and feed intake were measured weekly. Drinking-water consumption was determined twice per week. Ophthalmoscopy was performed before the start and at the end of the treatment period and at the end of the recovery period on all animals of the control and high dose groups. Clinicochemical and haematological examinations were performed after approximately 1, 2 and 3 months of test substance administration as well as towards the end of the recovery period. Urinalyses were performed after approximately 3 months of test substance administration and towards the end of the recovery period. Animals were sacrificed on completion of the 3-month treatment period and subjected to a full macroscopic pathological examination. Weights of selected organs were determined, and histopathological investigations were

Table 21. Body weights at the end of the administration and recovery periods in rats administered cycloxydim as the sodium salt

	Body weight (g)					
	Concentration in drinking-water (ppm)					
	0	30	100	300	900	2700
At end of administration period						
- Males	448.3	450.2	462.1	468.0	439.4	407.0** (90.8)[a]
- Females	258.7	247.2	261.8	250.0	263.0	254.5
At end of recovery period						
- Males	487.1	—	—	—	475.8	475.3
- Females	273.5	—	—	—	275.1	271.2

From Kuehborth et al. (1985)
** $P < 0.01$ (Dunnet's test)
[a] Percentage of control value.

performed. Animals of the recovery groups were also subjected to organ weight determinations as well as to gross pathological and histopathological examinations. Stability and homogeneity of the test substance were verified analytically. The correctness of the concentrations in the drinking-water and the stability in the water were analytically confirmed.

No mortality or clinical signs of toxicity occurred in the study at any dose level. There was a reduction of drinking-water consumption, especially during the earlier phases of treatment, in the high dose group males (−32%) and females (−35%) as well as in the 900 ppm females (−17%). However, towards the end of the administration period, drinking-water consumption in nearly all animals was comparable to that of controls. There were no effects on drinking-water consumption during the recovery period. A slight reduction of food consumption was observed in high dose group males (maximum −11%) throughout the administration period and in high dose group females (maximum −7%) during the initial phases of treatment. Males recovered completely during the treatment-free period. Body weights were reduced in high dose group males throughout the administration period. In high dose group females, there was a slight reduction in body weight development only during the first week of treatment. Thereafter, body weight development in all treated females was comparable to that of controls. There were no test substance–related differences in body weight between test groups in either sex at the end of the recovery period (Table 21).

There were no test substance–related ophthalmological findings in the groups. The haematological examinations and urinalysis did not show test substance–related changes. The clinicochemical investigations revealed a few marginal changes that were considered to be test substance related by the authors of the report (Tables 22 and 23). These are:

- increased alanine aminotransferase activity in 2700 and 900 ppm males and females (only after 1 month of treatment);
- increased creatinine level in 2700 and 900 ppm females;
- increased urea and cholesterol levels in 2700 ppm females (only after 1 month of treatment).

There were no clinicochemical changes in the recovery groups.

Organ weight determinations as well as gross pathological and histopathological examinations did not reveal any test substance–related changes at any dose level. Considering the above, it can be concluded that the high dose level of 2700 ppm resulted in a clear reduction in food consumption and body weight development. At this dose level and in 900 ppm females, there was also a reduction in

Table 22. Clinicochemical changes (after 1 month of treatment)

Parameter	Concentration in drinking-water (ppm)					
	0	30	100	300	900	2700
Alanine aminotransferase (µkat/l)						
- Males	0.89	0.94	0.93	0.92	0.97**	0.98**
- Females	0.92	0.92	0.99	0.98	1.02*	1.10**
Alkaline phosphatase (µkat/l)						
- Males	8.71	8.80	8.71	8.34	7.82*	7.83*
- Females	6.80	6.46	6.48	6.32	6.41	6.11
Creatinine (µmol/l)						
- Males	55.60	56.57	54.83	53.44	58.34*	56.41
- Females	50.32	50.93	50.31	51.20	53.77**	54.50**
Urea (mmol/l)						
- Males	6.56	6.72	6.61	6.49	6.45	6.52
- Females	7.61	7.98	7.40	7.53	8.05	8.38*
Cholesterol (mmol/l)						
- Males	1.65	1.73	1.56	1.53	1.75	1.69
- Females	1.44	1.37	1.43	1.50	1.60*	1.55

From Kuehborth et al. (1985)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

Table 23. Clinicochemical changes (at the end of the study)

Parameter	Concentration in drinking-water (ppm)					
	0	30	100	300	900	2700
Alanine aminotransferase (µkat/l)						
- Males	1.12	1.06	1.03	1.12	1.00	1.10
- Females	1.10	1.36	1.05	1.27	1.07	1.22*
Alkaline phosphatase (µkat/l)						
- Males	5.25	5.50	5.53	6.18	4.94	5.25
- Females	4.39	4.05	4.36	4.29	4.12	3.75**
Creatinine (µmol/l)						
- Males	53.37	55.79	52.48	57.02	55.56	55.26
- Females	57.83	55.12	55.63	57.85	59.07	59.38
Urea (mmol/l)						
- Males	6.86	7.05	6.83	6.90	6.58	6.79
- Females	7.56	8.11	8.13	8.06	7.76	7.91
Cholesterol (mmol/l)						
- Males	1.64	1.83	1.51	1.46	1.74	1.67
- Females	1.61	1.55	1.62	1.65	1.66	1.87*

From Kuehborth et al. (1985)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

Table 24. Test substance intake (free acid) by dogs administered cycloxydim sodium salt in the diet for 4 weeks

Nominal test substance intake (mg/kg bw per day)	Test substance intake in males (mg/kg bw per day)	Test substance intake in females (mg/kg bw per day)
10	9.9	9.6
40	39.8	39.4
120	118	119
360	356	338

From Hellwig et al. (1985)

water consumption. As the test substance was administered via the drinking-water, the reduction in water consumption is to be regarded as a palatability effect rather than a specific adverse effect.

Based on increases in creatinine, urea and cholesterol in females and increases in alanine aminotransferase activity in both sexes at 900 ppm (corresponding to intakes of 72 mg/kg bw per day in males and 74 mg/kg bw per day in females), the NOAEL was assessed to be 300 ppm (22 mg/kg bw per day in males and 28 mg/kg bw per day in females). GLP compliance certification was attached, and the study was conducted as per OECD test guideline 408 and USEPA guideline 82-1 (Kuehborth et al., 1985).

Dogs

Cycloxydim sodium salt (purity 94.8%; batch No. N 64 [Tox Batch II]) was administered via the diet for 4 weeks to two male and two female Beagle dogs (BASF's own breed) per dose level at concentrations of 0, 300, 1200, 3600 or 10 800 ppm to achieve a daily test substance intake of 0, 10, 40, 120 and 360 mg/kg bw per day (as free acid), respectively. The dietary preparation was made by mixing 350 g of dry dog food with 350 ml of water containing the appropriate concentration of cycloxydim sodium salt. The animals were offered the feed test substance preparation daily for 1 h in the morning. The feed was then removed from the kennel. For estimation of actual food consumption, any feed left over was weighed and subtracted from the amount offered. At the initiation of the study, the age of the animals was 6–8 months, and average weights were 8.9 kg and 8.2 kg for males and females, respectively.

Food consumption and the health status of the animals were checked daily, and body weights were determined once a week. Clinicochemical and haematological examinations were performed 5 days before the start of the administration period and on study days 9 and 24. Urinalyses were performed 6 days before the start of the administration period and on study days 15 and 22.

At the end of the 4-week administration period, all animals were sacrificed. Organ weights were determined, and gross pathological as well as histopathological examinations were carried out. The stability of the test substance was demonstrated for the stock and drinking-water solutions as well as for the formulations of feed/aqueous sodium salt solutions. The stability of the test substance in feed demonstrated an average recovery of more than 90% within the first hour. After 3 h, the recovery ranged between 76.5% and 80.1%. As the dietary preparations were offered to the dogs immediately after preparation and the diets were made available to the dogs only for 1 h, the limited stability is considered not to have affected the intake of unchanged test substance. The correctness of the concentration in the drinking-water was analytically verified, with only a single exception in a single dose group. This is not considered to have had an impact on the results of the study.

The overall mean test substance intake for the 4-week treatment period was calculated based on the weekly test substance intake data provided in the report (Table 24).

There were no mortalities in any of the groups. A single occurrence of vomiting in a high dose group female could be regarded as a possible treatment-related sign of clinical toxicity. Food

Table 25. Haematological and clinicochemical parameters at day 24 in dogs administered cycloxydim sodium salt in the diet

Parameter	Dose level (mg/kg bw per day)				
	0	10	40	120	360
Haemoglobin (mmol/l)					
- Male 1 / Male 2	9.70 / 9.17	7.93 / 8.05	8.55 / 8.27	8.47 / 8.18	7.01 / 8.05
- Female 1 / Female 2	8.91 / 9.53	8.79 / 9.72	10.18 / 9.98	9.14 / 8.88	9.35 / 7.79
Red blood cell count (10^{12}/l)					
- Male 1 / Male 2	7.68 / 7.14	6.26 / 6.37	6.67 / 6.27	6.56 / 6.17	5.27 / 6.06
- Female 1 / Female 2	6.54 / 7.25	6.61 / 7.42	7.67 / 7.51	6.90 / 7.74	7.27 / 6.41
Haematocrit (l/l)					
- Male 1 / Male 2	0.52 / 0.46	0.42 / 0.43	0.45 / 0.43	0.46 / 0.42	0.37 / 0.43
- Female 1 / Female 2	0.47 / 0.51	0.47 / 0.52	0.53 / 0.53	0.48 / 0.47	0.50 / 0.44
Heinz bodies (‰)					
- Male 1 / Male 2	4 / 3	11 / 2	4 / 10	6 / 11	30 / 38
- Female 1 / Female 2	10 / 10	4 / 5	14 / 8	7 / 12	10 / 33
Alkaline phosphatase (µkat/l)					
- Male 1 / Male 2	5.01 / 5.05	5.71 / 6.33	4.90 / 4.16	8.38 / 7.69	10.8 / 16.3
- Female1 / Female 2	3.38 / 5.56	4.22 / 8.42	5.90 / 8.17	4.20 / 6.49	9.00 / 11.1

From Hellwig et al. (1985)

consumption was slightly reduced in the two high dose group females, which is also apparent from the slightly reduced actual test substance intake values compared with the nominal concentration. Body weight gain was not affected in any of the treatment groups. The haematological investigations showed a marginal reduction in the red blood cell count, haematocrit and haemoglobin values in the high dose group males as well as an increased occurrence of Heinz bodies both in males and in one female dog administered 360 mg/kg bw per day. Clinicochemical examinations revealed an increase in alkaline phosphatase activity in males and females of the 360 mg/kg bw per day group as well as in males of the 120 mg/kg bw per day group (Table 25). In addition, the plasma cholinesterase activity was increased in high-dose males; however, this was not considered to represent any toxicologically significant effect and is likely to be an incidental finding.

Organ weight determinations revealed a clear increase in liver weights at 360 mg/kg bw per day and slight liver weight increases in the 40 and 120 mg/kg bw per day groups (Table 26).

Gross necropsy and histopathological investigations did not show any test substance–related changes. In the absence of histopathological liver changes up to the highest dose tested, the increase in relative liver weights is considered to represent an adaptive response rather than an adverse effect.

From the above, it can be concluded that the high dose level of 360 mg/kg bw per day was reasonably well tolerated; there was a single female with vomiting, and there was a slight reduction in food consumption in males. Red blood cell parameters were marginally reduced in high dose group males and in one male of the 120 mg/kg bw per day group. Clinicochemical examinations revealed an increase in alkaline phosphatase activity in males at 120 mg/kg bw per day and in both sexes at 360 mg/kg bw per day. Liver weights were slightly increased at 40 and 120 mg/kg bw per day and clearly elevated at 360 mg/kg bw per day. No evidence of histopathological liver changes was observed up to the highest dose level tested.

The NOAEL in this study is 40 mg/kg bw per day (actual dose) in males and 119 mg/kg bw per day (actual dose) in females, on the basis of effects on the liver. A QA statement was attached (Hellwig et al., 1985).

Table 26. Terminal body and liver weights in dogs administered cycloxydim sodium salt in the diet for 4 weeks

Parameter	Dose level (mg/kg bw per day)									
	0		10		40		120		360	
Terminal body weight, g (% of control)										
- Males	8700	(—)	9150	(105)	10 250	(118)	9900	(114)	9900	(114)
- Females	8450	(—)	8650	(102)	9 600	(114)	9450	(112)	9150	(108)
Absolute liver weight, g (% of control)										
- Males	322.79	(—)	351.00	(109)	407.45	(126)	415.01	(129)	532.74	(165)
- Females	317.62	(—)	341.83	(108)	397.74	(125)	410.49	(129)	464.98	(146)
Relative liver weight, % (% of control)										
- Males	3.713	(—)	3.841	(103)	4.040	(109)	4.196	(113)	5.371	(145)
- Females	3.751	(—)	3.954	(105)	4.143	(110)	4.336	(116)	5.089	(136)

From Hellwig et al. (1985)

In a 90-day study, cycloxydim (purity 94.8%; batch No. N 64) was administered as the sodium salt to groups of four male and four female Beagle dogs via their diet. The dose levels were 0, 60, 300, 1500 and 7500 ppm (equivalent to 0, 2, 10, 50 and 250 mg/kg bw per day). The diet was prepared freshly each day by mixing 350 g of dry dog food with 350 ml of drinking-water containing the appropriate concentration of cycloxydim sodium salt. The animals were offered the feed/test substance preparation daily for 1 h in the morning. The feed was then removed from the kennel. For estimation of actual feed consumption, any feed left over was weighed and subtracted from the amount offered. This procedure was followed in order to ensure sufficient stability of the test substance in the feed preparation. At the initiation of study, the ages of the dogs were 6–9 months, with average weights of 10.4 kg for males and 9.1 kg for females.

The food consumption of the animals was determined daily, and their body weight was measured once a week; the dogs' health was checked each day. Clinicochemical and haematological examinations as well as urinalyses were carried out once prior to the start of the administration period and after approximately 1 month and 3 months of treatment. Ophthalmological examinations were carried out before the beginning of the study and towards the end of the administration period in the animals of the high dose group and the controls. All animals were assessed gross pathologically. The liver, kidneys, testes, thyroids and adrenal glands were weighed. All gross lesions and the livers from all animals under study were histopathologically examined. Furthermore, a comprehensive set of organs from the control, 1500 and 7500 ppm dose groups was subjected to histopathological examination.

There were no mortalities or signs of clinical toxicity in any of the groups. Food consumption and body weight development were not affected at any dose level. The haematological investigations showed, at the high dose level only, a reduction in red blood cell count for both sexes and an increase in platelet count. The number of Heinz bodies was increased in all four high dose group females and in one high dose group male. In high dose group males only, increases in reticulocytes (only at the 1-month sampling time point), polymorphonuclear neutrophilic granulocytes, and mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) values were observed (Table 27).

In clinicochemical examinations, a 2- to 3-fold increase in alkaline phosphatase activity was observed in high dose group males and females. A doubtful (statistically non-significant) increase

Table 27. Haematology results at end of study for dogs administered cycloxydim sodium salt in the diet for 3 months

Parameter	Dietary concentrations (ppm)				
	0	60	300	1500	7500
Red blood cells (10^{12}/l)					
- Males	7.64	7.16	7.38	7.63	6.89*
- Females	7.66	7.39	7.81	7.65	7.02**
MCV (fl)					
- Males	68.10	69.58	67.60	68.53	71.78*
- Females	69.42	69.72	69.47	70.50	70.40
MCH (fmol)					
- Males	1.328	1.334	1.317	1.328	1.383*
- Females	1.330	1.336	1.329	1.320	1.351
Reticulocytes (‰)					
- Males (1 month)	3.50	3.00	1.75	2.25	9.25
- Males (3 months)	6.75	4.25	1.00	3.25	5.50
- Females (1 month)	5.0	5.25	2.25	3.25	2.75
- Females (3 months)	0.75	3.25	2.25	4.75	2.67
Heinz bodies (‰)					
- Male No. 1/2/3/4	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/1/9
- Female No. 1/2/3/4	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	9/7/10/8
Platelets (10^9/l)					
- Males	462.50	442.75	457.25	402.75	554.00*
- Females	426.50	487.00	461.75	392.25	647.25**
Polymorphonuclear neutrophilic granulocytes (10^9/l)					
- Males	4.83	5.56	6.03	6.55	7.97
- Females	6.01	5.68	5.90	6.49	6.31

From Hellwig et al. (1986)
* $P < 0.05$; ** $P < 0.01$ (t-test)

in the mean alkaline phosphatase activity was seen in males at 1500 ppm, which resulted from a high value of dog no. 1 (7.95 µkat/l); the other three males of the group showed values that were comparable with control levels (range 3.16–3.60 µkat/l). No effects on alkaline phosphatase activity were seen in 1500 ppm females. The albumin concentration was decreased in both sexes at the high dose level only, accompanied by an increased globulin concentration in high dose group females; the total protein content was unaffected by treatment. Furthermore, slightly decreased potassium concentrations were seen in males at 7500 ppm (Table 28).

Organ weight determination revealed a statistically significant increase in absolute and relative liver weights in high-dose males and females, as well as a statistically significant increase in absolute and relative thyroid weights in high-dose males (Table 29).

Gross pathological investigations showed a reddish tinge to the bile for high-dose males and females only. Histopathology revealed an enlargement of hepatocytes in three out of four males and in all females at the high dose level only. There were no other test substance–related changes noted in the histopathological examinations. There were no test substance–related findings at 1500, 300 or 60 ppm.

Table 28. Clinical chemistry results at end of study for dogs administered cycloxydim sodium salt in the diet for 3 months

Parameter	Dietary concentration (ppm)									
	0		60		300		1500		7500	
Alkaline phosphatase, µkat/l (% of control)										
- Males	3.03	(—)	3.36	(111)	3.11	(102)	4.83	(159)	9.46**	(312)
- Females	4.64	(—)	3.28	(71)	3.88	(84)	4.54	(98)	10.04**	(216)
Albumin, g/l (% of control)										
- Males	40.20	(—)	37.30	(93)	38.37	(95)	40.12	(100)	35.32*	(88)
- Females	40.45	(—)	40.73	(101)	39.90	(99)	38.27	(95)	34.45**	(85)
Globulin, g/l (% of control)										
- Males	20.93	(—)	20.08	(96)	20.79	(99.33)	22.18	(106)	23.15	(110)
- Females	19.22	(—)	21.09	(110)	20.74	(108)	19.10	(99)	23.77**	(124)
Potassium, mmol/l (% of control)										
- Males	4.07	(—)	4.10	(101)	3.94	(97)	3.81	(94)	3.64*	(89)
- Females	3.92	(—)	3.90	(99.48)	3.99	(101.78)	3.73	(95.15)	3.75	(95.66)

From Hellwig et al. (1986)
* $P < 0.05$; ** $P < 0.01$ (*t*-test)

Table 29. Organ weights in dogs administered cycloxydim sodium salt in the diet for 3 months

Parameter	Dietary concentration (ppm)									
	0		60		300		1500		7500	
Absolute liver weight, g (% of control)										
- Males	344	(—)	365	(106)	363	(106)	387	(113)	479**	(139)
- Females	316	(—)	316	(100)	337	(107)	361	(114)	498**	(158)
Relative liver weight, % (% of control)										
- Males	3.01	(—)	3.18	(106)	3.24	(108)	3.36	(112)	4.13**	(137)
- Females	3.07	(—)	3.21	(104)	3.05	(99)	3.29	(107)	4.75**	(155)
Absolute thyroid weight, g (% of control)										
- Males	0.716	(—)	0.774	(108)	0.859	(120)	0.865	(121)	1.060*	(148)
- Females	0.849	(—)	0.674	(79)	0.771	(91)	0.812	(96)	1.079	(127)
Relative thyroid weight, % (% of control)										
- Males	0.0062	(—)	0.0068	(110)	0.0077	(124)	0.0075	(121)	0.009*	(145)
- Females	0.0082	(—)	0.0069	(84)	0.007	(85)	0.0075	(91)	0.010	(127)

From Hellwig et al. (1986)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

To conclude, the 3-month administration of cycloxydim to dogs via the feed did not result in clinical findings or body weight changes up to the highest dose tested of 7500 ppm. At this dose level, there were signs of an anaemic process with compensatory reactions. The target organ was the liver, with increased weights and hepatocellular hypertrophy; the bile had a reddish colour. Most of the clinicochemical changes were also considered to be related to liver changes. Thyroid weights were also significantly increased in males at 7500 ppm and more than 120% increased at dose levels of 300 and 1500 ppm (not statistically significant); however, no associated histopathological changes of the thyroid were observed. Findings at this dose are considered either not treatment related or not adverse. Slight increases in alkaline phosphatase activity and liver weight (+12%) (for liver) were not statistically significant and not associated with histopathological liver changes. The slight (non-significant) thyroid weight increase was not considered treatment related because it was not reproducible in 4-week or 1-year dog studies; no histopathological changes were seen in dog thyroid. Further, in male dogs, liver weights were increased over 110% at a concentration of 1500 ppm; this effect was not statistically significant, but coincided with increases in alkaline phosphatase activity (159% of control value).

Considering the changes in haematological parameters and liver effects, the NOAEL in this study was 1500 ppm, equal to 50 mg/kg bw per day. It is certified by the laboratory that the test facility was inspected by the competent authority in accordance with the OECD guidelines for national GLP inspection and study audits regarding compliance with the principles of GLP. The study was conducted as per OECD test guideline 409 and USEPA guideline 82-1 (Hellwig et al., 1986).

Cycloxydim (purity 93.9%; batch No. N 88) as the sodium salt was administered to six male and six female Beagle dogs per group at dietary dose levels of 0, 400, 1600 and 6400 ppm (equal to 0, 12, 49 and 206 mg/kg bw per day as the acid) for 12 months. The diets were prepared each day by mixing 350 g of pulverized dog food with 350 g of water containing the appropriate amount of the sodium salt of cycloxydim. Food consumption was determined daily, and body weight was determined once a week. The animals' health was checked each day. The dogs were approximately 6–8 months old at the beginning of the study, and the average body weights were 9.7 kg and 9.2 kg for males and females, respectively.

Clinicochemical and haematological examinations as well as urinalyses were carried out prior to the start of treatment and after approximately 3, 6 and 12 months of administration. Ophthalmological examinations were carried out before the start of the study and towards the end of the administration period. All animals were assessed gross pathologically; organ weights were determined, and organs were subsequently subjected to a complete histopathological examination as specified in the OECD guideline.

There were no test substance–related signs of clinical toxicity or mortalities in any group. Food consumption and body weight development were not adversely affected in any treatment group. The haematological investigations (Table 30) demonstrated the following slight changes, which were confined, with two exceptions, to the high dose level and generally observable only at the 3- and 6-month time points:

6400 ppm:

- decreased erythrocytes—males (6 months) and females (3 and 6 months)
- decreased haemoglobin and haematocrit—females (3 and 6 months)
- increased MCH—males (3 months), females (3 and 6 months)
- increased MCV—males (3 months), females (3, 6 and 12 months)
- increased platelets and Heinz bodies—males and females (3, 6 and 12 months).

Table 30. Haematological parameters after 3, 6 and 12 months of dietary administration of cycloxydim sodium salt to dogs

Parameter	3 months				6 months				12 months			
	Dietary concentration (ppm)											
	0	400	1600	6400	0	400	1600	6400	0	400	1600	6400
Males												
RBC (10^{12}/l)	6.56	6.23	6.36	6.43	6.61	6.26	6.23	6.19*	7.08	7.12	6.85	6.90
Hb (mmol/l)	9.53	9.12	9.46	9.62	9.75	9.25	9.27	9.35	10.25	10.34	10.06	10.20
Ht (l/l)	0.457	0.434	0.452	0.463	0.467	0.443	0.446	0.451	0.500	0.500	0.485	0.496
MCH (fmol)	1.45	1.46	1.49	1.50*	1.48	1.48	1.49	1.51	1.45	1.45	1.47	1.48
MCV (fl)	69.55	69.52	70.88	71.95*	70.68	70.67	71.43	72.73	70.37	70.23	70.68	71.73
Platelets (10^9/l)	460	516	528	583**	450	450	513*	532	471	464	521	589**
Heinz bodies (‰)	4	8	12	21**	2	3	9**	25**	3	2	5	29**
Females												
RBC (10^{12}/l)	6.84	6.63	6.75	5.90**	6.78	6.64	6.66	5.83*	6.67	6.74	7.04	6.46
Hb (mmol/l)	10.01	9.78	10.03	8.97*	9.96	9.79	9.88	8.89*	9.62	9.68	10.14	9.56
Ht (l/l)	0.477	0.460	0.477	0.430*	0.478	0.469	0.477	0.426*	0.458	0.470	0.499	0.467
MCH (fmol)	1.46	1.48	1.48	1.52*	1.47	1.47	1.48	1.52**	1.45	1.44	1.44	1.48
MCV (fl)	69.63	69.23	70.45	72.70**	70.45	70.58	71.53	72.95*	68.63	69.60	70.72	72.20*
Platelets (10^9/l)	524	452	541	712*	469	467	543	699*	576	464	517	703
Heinz bodies (‰)	3	3	3	24**	3	2	7*	27**	5	9	13**	41**

From Hellwig & Hildebrand (1988)
Hb, haemoglobin; Ht, haematocrit; RBC, red blood cells; * $P < 0.05$; ** $P < 0.02$ (Kruskal-Wallis ANOVA and Mann-Whitney *u*-test, two-sided)

1600 ppm:

- increased Heinz bodies—males (3 months, marginal effect; 6 months, significant effect), females (6 and 12 months).

The clinicochemical investigation (Table 31) demonstrated the following changes, most of which were already expressed after 3 months of treatment and did not alter during the course of the study:

- increased alkaline phosphatase activity: 6400 ppm (both sexes) and 1600 ppm (males)
- decreased total protein concentration: 6400 ppm (males)
- decreased albumin concentration: 6400 ppm (both sexes) and 1600 ppm (males).

Urinalysis and ophthalmoscopy did not demonstrate test substance–related effects. Organ weight determinations revealed an increase in absolute and relative liver weights in the 6400 ppm males and females, as well as in the 1600 ppm males (Table 32).

Histopathology demonstrated slight or moderate haemosiderosis in the livers of three females of the 6400 ppm group. This effect is considered to have resulted from the transiently increased degradation of erythrocytes induced by the test substance. There were no further test substance–related changes noted during the histopathological investigations. In particular, there were no changes in the liver that could be associated with the increased liver weights.

In view of the above, it can be concluded that a dose level of 6400 ppm induced an anaemic effect in Beagle dogs, which showed signs of reversibility during the last 6 months of treatment.

Table 31. Clinicochemical parameters after 3, 6 and 12 months of dietary administration of cycloxydim sodium salt to dogs

Parameter	3 months				6 months				12 months			
	Dietary concentration (ppm)											
	0	400	1600	6400	0	400	1600	6400	0	400	1600	6400
Males												
AP (µkat/l)	3.73	4.93	6.11**	10.64**	2.96	3.82	4.54*	9.38**	3.03	4.34	5.47**	11.65**
Total protein (g/l)	56.85	54.87	56.77	53.54	59.40	58.24	57.20	54.38*	60.12	58.96	58.10	56.03
Albumin (g/l)	35.76	34.01	33.99	30.90**	39.84	38.71	37.52**	33.77**	39.92	38.09	36.56**	33.84**
Females												
AP (µkat/l)	4.00	4.61	4.90	9.93**	2.91	4.07	3.96	8.99**	5.83	4.70	4.55	9.53
Total protein (g/l)	56.29	57.95	55.95	53.63	57.13	57.33	56.81	55.72	58.57	60.30	60.07	56.83
Albumin (g/l)	34.81	35.17	33.65	30.23**	38.75	39.70	37.71	34.14**	36.73	38.29	37.42	33.98

From Hellwig & Hildebrand (1988)
AP, alkaline phosphatase; * $P < 0.05$; ** $P < 0.02$ (Kruskal-Wallis ANOVA and Mann-Whitney *u*-test, two-sided)

Table 32. Terminal body weights and liver weights in dogs administered cycloxydim sodium salt in the diet for 1 year

Parameter	Dietary concentration (ppm)							
	0		400		1600		6400	
Terminal body weight, kg (% of control)								
- Males	11.92	(—)	12.70	(107)	11.53	(97)	11.53	(97)
- Females	10.87	(—)	11.32	(104)	12.12	(111)	10.63**	(98)
Absolute liver weight, g (% of control)								
- Males	349	(—)	385	(110)	407*	(117)	449**	(129)
- Females	381	(—)	414	(109)	384	(101)	457*	(120)
Relative liver weight, % (% of control)								
- Males	2.94	(—)	3.04	(103)	3.54*	(120)	3.93**	(134)
- Females	3.51	(—)	3.67	(105)	3.17	(90)	4.29**	(122)

From Hellwig & Hildebrand (1988)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test)

Associated with the anaemia, histopathology demonstrated increased haemosiderosis in the livers of females of the 6400 ppm group. Liver weights were increased at this dose level.

The NOAEL in this 12-month dietary study in dogs was 400 ppm (equal to 12 mg/kg bw per day) for males on the basis of effects on erythrocytes and the liver and altered clinicochemical parameters at 1600 ppm. The study complied with GLP, and a QA statement was attached (Hellwig & Hildebrand, 1988).

(b) Dermal route

Rats

Cycloxydim (purity 92.9%; batch No. WH 16884) was applied to the skin of 10 male and 10 female Wistar rats per dose level on an area corresponding to at least 10% of the body surface for

a period of 6 h/day, 5 days/week, for 4 consecutive weeks. The doses were 0 (vehicle control, olive oil), 60, 300 and 1000 mg/kg bw per day. The administration volume was 2 ml/kg bw per day. The skin was covered by a semiocclusive dressing. After removal of the dressing, the skin was washed with lukewarm water. At the start of the study, the rats were 60 ± 1 days old, and the body weights were in the range of 174.7–215.5 g (group mean 195.8 g) for males and 146.4–192.8 g (group mean 167.9 g) for females.

The animals were examined at least once a day for clinical signs and mortalities. Additional clinical examinations, including detailed examinations of the skin, were carried out daily. Detailed clinical observations in an open field were conducted prior to the start of the administration period and weekly thereafter. An FOB was performed towards the end of the study. A motor activity measurement was performed on the same day as the FOB. Food consumption and body weight were determined weekly. Clinicochemical and haematological examinations as well as urinalysis were performed at the end of the administration period. Ophthalmological examinations were carried out prior to the start and towards the end of the administration period. All animals were subjected to a gross pathological assessment followed by histopathological examinations.

There were no mortalities or clinical signs of toxicity in the treatment groups. No substance-related effects on food consumption were observed in any treatment group. Food efficiency was statistically significantly decreased in males at 1000 mg/kg bw per day on day 21. A relationship to treatment cannot be excluded with certainty. In females, food efficiency was statistically significantly decreased on day 21 at 60 and 300 mg/kg bw per day, but not at 1000 mg/kg bw per day. Owing to the lack of a dose–response relationship, this was assessed as being incidental and not treatment related. No statistically significant deviations were seen concerning body weight. A statistically significantly lower body weight gain was observed in high dose group males for the 4-week study period (−22% compared with controls). The mean terminal body weight in males at 1000 mg/kg bw per day was decreased by 6% (statistically non-significant) compared with the control group. Body weight change was also statistically significantly decreased in females at 60 and 300 mg/kg bw per day on day 21. However, owing to the lack of a dose–response relationship, this was assessed as being incidental and not treatment related. No statistically significant changes in body weight development were noted in treatment group females when based on the 4-week treatment period, and no effects on terminal body weight were seen that could be considered as substance related (Table 33).

Open field observations, FOB and motor activity determinations did not show test substance–related changes. Clinicochemical and haematological examinations and urinalysis did not reveal test substance–related changes. Gross macroscopic observations and histopathological examinations did not show any treatment-related effect. There were no signs of skin irritation in any of the animals.

The NOAEL for systemic toxicity is 1000 mg/kg bw per day for females (highest dose tested) and 300 mg/kg bw per day for males, based on slight effects on body weight gain and food efficiency at 1000 mg/kg bw per day. Signs of local skin irritation were not observed. The study has been conducted as per GLP and the following guidelines: European Economic Community (EEC) 92/69, OECD 410 and USEPA 870.3200 (Mellert et al., 2001a).

2.3 Long-term studies of toxicity and carcinogenicity

Long-term studies of oral toxicity in mice and rats were conducted using cycloxydim sodium salt or cycloxydim free acid. Cycloxydim sodium salt as test substance was applied instead of cycloxydim free acid if the free acid could not be used in the feed because of its insufficient stability and could also not be administered in the drinking-water on account of its low solubility.

In rodent long-term toxicity studies, cycloxydim sodium salt was administered via the drinking-water after it was found that the sodium salt was not sufficiently stable when mixed in rodent

Table 33. Terminal body weight and body weight gain in rats dermally administered cycloxydim for 4 weeks

Parameter	Dose level (mg/kg bw per day)							
	0		60		300		1000	
Males								
Food efficiency (day 21) (% of control)	10.6	(—)	8.9	(84)	9.3	(88)	7.1*	(67)
Body weight change (days 0–21), g (% of control)	65.6	(—)	60.6	(92)	70.2	(107)	54.0	(82)
Body weight change (days 0–27), g (% of control)	66.7	(—)	60.9	(91)	69.9	(105)	52.2*	(78)
Terminal body weight, g (% of control)	262.4	(—)	256.3	(98)	266.8	(102)	247.3	(94)
Females								
Food efficiency (day 21) (% of control)	11.9	(—)	5.1*	(43)	3.6**	(30)	7.7	(65)
Body weight change (days 0–21), g (% of control)	41.5	(—)	31.2*	(75)	31.9*	(77)	36.9	(89)
Body weight change (days 0–27), g (% of control)	41.6	(—)	37.9	(91)	36.0	(87)	40.5	(97)
Terminal body weight, g (% of control)	211.8	(—)	204.4	(97)	203.7	(96)	207.7	(98)

From Mellert et al. (2001a)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

feed. After only 1 day, the active ingredient concentration in the mixture of feed and test substance was only about 78% of the initial value, and after 8 days, only about 50% was detected.

In all studies, the feed concentration values (in ppm) and test substance intakes (in mg/kg bw per day) refer to cycloxydim as the acid rather than as the sodium salt. Continuous test substance administration in these studies was verified by regular determination of the consumption of drinking-water containing the test substance (2 times per week).

The results of these studies are characterized by clinicochemical changes, associated with changes in water and food consumption, and effects on the liver. Where the test substance was administered in the drinking-water, the reduction in water consumption is regarded to be a palatability effect rather than a specific adverse effect.

Mice

Cycloxydim (purity 93.9%; batch No. N 88) was administered as the sodium salt to 50 male and 50 female B6C3F1 mice per dose level for 24 months. The concentrations in the drinking-water were 10, 20, 60 and 240 ppm (equal to 1.3, 3.0, 8.4 and 32 mg/kg bw per day as free acid). Dose levels were selected based on the results of a 4-week feeding study in mice, in which increased liver weights were observed at concentrations of 100 ppm and above. Two groups of untreated controls were maintained in parallel for comparison, each with 50 males and 50 females. At the beginning of the administration of the test substance, the mice were 49 days old, and their mean body weights were 23 g and 20 g for males and females, respectively.

Drinking-water consumption and body weight were determined once a week. Food consumption was not measured. The animals' health was checked at least daily. Detailed clinical examinations were performed once a week. At the end of the administration period, differential blood counts were determined in all surviving animals of the control and high dose groups. Differential blood counts were also determined in all animals that died during the study. After 24 months of administration,

all animals were assessed by gross pathology and histopathology. Organ weights of selected organs were determined.

There were neither test substance–related increased mortalities nor clinical signs of toxicity. Body weight and body weight gain were not affected at any dose level. There were no effects on drinking-water consumption. There were no test substance–related changes found in the examination of the differential blood counts at any dose level. There were no test substance–related changes in organ weights. Gross pathological and histopathological examinations did not show test substance–related effects at any dose level in any dose group. There was no test substance–related increase in the incidence of any neoplasia in any of the satellite groups.

As there were no test substance–related changes at any dose level, the NOAEL in this study was 240 ppm, equal to 32 mg/kg bw per day for males and females (i.e. the highest dose tested). The study was not adequate for the evaluation of carcinogenicity, as the doses delivered were not sufficiently high; the highest dose used was much less than the NOAEL of 1000 ppm identified in the dose range–finding studies. Therefore, it can be concluded that cycloxydim had not been adequately tested in mice. The study complied with GLP, and a QA statement was attached (Kuehborth et al., 1988b).

Rats

Cycloxydim (purity 93.9%; batch No. N 88) was administered for 18 months in the drinking-water as the sodium salt to 20 male and 20 female Wistar rats per dose level in a chronic toxicity study. The dose levels in the drinking-water were 0, 100, 400, 1600 and 2700 ppm (equal to 0, 7, 28, 103 and 171 mg/kg bw per day). The rats were 42 days old when substance administration began, and the average body weights were 197 g and 150 g for males and females, respectively.

Food consumption was determined once a week for the first 14 weeks. Thereafter, it was determined at 3-month intervals. Body weights and drinking-water consumption were determined once per week for the entire administration period. The animals' health was checked each day. Detailed clinical examinations were performed once a week. Ophthalmological examinations were carried out before the start of the study and after 3, 6, 12 and 18 months of administration in the animals of the control and high dose groups. Clinicochemical and haematological examinations as well as urinalyses were carried out after about 3, 6, 12 and 18 months of administration. After 18 months of administration, all animals were assessed by gross pathology and histopathology. Organ weights of selected organs were determined.

There were no test substance–related mortalities or signs of clinical toxicity in any of the treatment groups. Food consumption was reduced by approximately 8–9% throughout the study in both sexes at 2700 ppm and in males at 1600 ppm compared with the control group. Food consumption in the 400 and 100 ppm treatment groups was comparable to the amount consumed by the controls. Drinking-water consumption was reduced in males and females of the 2700 ppm and 1600 ppm groups. In males, the reduction in drinking-water consumption was more pronounced during the early phases of the study (2 months: 2700 ppm, 80% and 76% of control level for males and females, respectively; 1600 ppm, 81.5% and 87.5% for males and females, respectively; 18 months: 2700 ppm, 94.5% and 74% for males and females, respectively; 1600 ppm, 94.5% and 80.2% for males and females, respectively).

After 18 months of treatment, body weights (Table 34) and body weight gains (Table 35) were reduced in males and females of the 2700 ppm, 1600 ppm and 400 ppm groups compared with the respective control values.

There were no haematological changes that could be related to the administration of the test substance. Clinicochemical examinations revealed a reduction in triglycerides in females of the 2700 ppm, 1600 ppm and 400 ppm groups. This reduction was noted in all of the blood samples taken after 3, 6, 12 and 18 months. The statistically significant decrease in triglycerides noted in females at 100 ppm after 18 months of treatment was considered to have resulted from an exceptionally

Table 34. Body weight after 18 months (day 546) in rats administered cycloxydim sodium salt in the drinking-water

Concentration in drinking-water (ppm)	Male body weight		Female body weight	
	g	% of control	g	% of control
0	749.9	—	399.0	—
100	711.3	95	380.4	95
400	678.7*	91	357.3*	90
1600	617.9**	82	319.7**	80
2700	589.5**	79	314.1**	79

From Kuehborth et al. (1988c)
* $P < 0.05$; ** $P < 0.01$ (ANOVA + Dunnett's tests)

Table 35. Body weight gain within 18 months (days 0–546) in rats administered cycloxydim sodium salt in the drinking-water

Concentration in drinking-water (ppm)	Male body weight gain		Female body weight gain	
	g	% of control	g	% of control
0	553.5	—	247.9	—
100	513.6	93	230.2	93
400	482.2	87	207.1	84
1600	422.2	76	169.5	68
2700	393.2	71	163.9	66

From Kuehborth et al. (1988c)

high control group value. As no corresponding decrease in triglyceride concentration was found at 100 ppm in the 24-month carcinogenicity study (Kuehborth et al., 1988a), a substance-related effect at 100 ppm was excluded. In males, a statistically significant decrease in triglyceride concentration was observed only in the 1600 ppm dose group after 12 and 18 months of treatment (Table 36). This effect was due to decreased triglyceride values in individual animals. In the absence of a dose–response relationship, it was concluded that triglycerides were not affected by treatment in males.

Urinalysis did not reveal any test substance–related changes. There were no ophthalmological changes related to the administration of the test substance. Organ weight determinations revealed no test substance–related changes. Gross pathological examinations did not show test substance–related effects at any dose level. In the liver, bile duct proliferation was more frequently recorded in treated males at 1600 and 2700 ppm than in controls, but the incidence was within the historical control range for this age-associated lesion. The incidence across the increasing dose groups was 20% (control), 20%, 15%, 35% and 40% in males and 0%, 5%, 5%, 10% and 0% in females. The relative number of males with basophilic foci in liver tissue was 10% (control), 5%, 35%, 10% and 10%, indicating no dose–response relationship for the occurrence of basophilic foci. Microscopic findings in other organs were found to be not treatment related.

In view of the above, it can be concluded that the administration of cycloxydim via the drinking-water resulted in a reduction of body weight gain in both sexes at concentrations of 400 ppm and above. Drinking-water consumption was reduced in the 1600 ppm and 2700 ppm groups. In females, there was a reduction in triglycerides at concentrations of 400 ppm and higher.

The NOAEL in this study was 100 ppm, equal to 7 mg/kg bw per day, in males and females based on a statistically significant reduction in body weight, body weight gain and triglyceride

Table 36. Triglyceride concentrations at 3, 6, 12 and 18 months in rats administered cycloxydim sodium salt in the drinking-water

Concentration in drinking-water (ppm)	Triglyceride concentration							
	3 months		6 months		12 months		18 months	
	mmol/l	% of control	mmol/l	% of control	mmol/l	% of control	mmol/l	% of control
Males								
0	3.67	—	4.90	—	5.24	—	6.28	—
100	3.95	108	5.52	113	6.15	117	5.82	93
400	4.29	117	5.52	113	5.20	99	6.04	96
1600	2.75	75	3.24	66	2.92**	56	3.75*	60
2700	3.45	94	3.99	81	4.05	77	5.03	80
Females								
0	3.74	—	5.27	—	5.89	—	7.82	—
100	3.45	92	4.16	79	5.06	86	5.13**	66
400	2.18*	58	3.30*	63	3.05**	52	4.59**	59
1600	2.68	72	2.73**	52	3.40**	58	4.24**	54
2700	2.02*	54	2.71**	51	2.77**	47	2.59**	33

From Kuehborth et al. (1988c)
* $P < 0.05$; ** $P < 0.01$ (t-test)

concentrations at a drinking-water concentration of 400 ppm, equal to 28 mg/kg bw per day. The study complied with GLP, and a QA statement was attached (Kuehborth et al., 1988c).

In a 2-year study, cycloxydim (purity 93.9%; batch No. N 88) was administered in the drinking-water as the sodium salt to 50 male and 50 female Wistar rats per dose level. The treatment levels in the drinking-water were 100, 400 and 1600 ppm (equal to 6.4, 26.4 and 99.2 mg/kg bw per day as free acid). The controls consisted of 100 males and 100 females. The rats were 42 days old when the test substance administration was initiated, and the body weights of the rats were 196 g and 155 g for males and females, respectively.

Food consumption was not determined; however, as there were no effects in the chronic study in rats (Kuehborth et al., 1988c), this does not affect the quality of the study. Body weight and drinking-water consumption were determined once per week for the entire administration period. The animals' health was checked each day. Detailed clinical examinations were performed once a week. Clinicochemical and haematological examinations as well as urinalysis were carried out on 10 animals of each sex per dose group towards the end of the administration period. In addition, creatinine, triglycerides and cholesterol levels were determined for all surviving animals at the end of the treatment period. After 24 months of administration, all animals were assessed for gross pathology and histopathology. Organ weights of selected organs were determined.

There were no test substance–related mortalities or signs of clinical toxicity in any of the treatment groups. Drinking-water consumption was reduced in males and females of the 1600 ppm group. In females, the extent of the reduction of drinking-water consumption was slightly more pronounced during the early phases of the study (Table 37).

Body weight gain was reduced in males and females of the 1600 ppm group. At a concentration of 400 ppm, the reduction in body weight was statistically significant only during the first year of treatment (Table 38).

Table 37. Water consumption by rats administered cycloxydim sodium salt in the drinking-water for 2 years

Concentration in drinking-water (ppm)	Water consumption											
	0–3 months		0–6 months		0–9 months		0–12 months		0–18 months		0–24 months	
	g/day	% of control	g/day	% of control	g/day	% of control	g/day	% of control	g/day	% of control	g/day	% of control
Males												
0	28.1	—	28.4	—	28.2	—	28.4	—	29.9	—	32.3	—
100	27.6	98	28.1	99	27.7	98	27.8	98	29.0	97	31.4	97
400	27.2	97	27.7	97	27.4	97	27.6	97	29.0	97	31.2	97
1600	25.4	90	26.2	92	25.7	91	25.3	89	27.0	90	29.4	91
Females												
0	23.6	—	25.5	—	26.1	—	27.1	—	29.2	—	31.1	—
100	23.4	99	24.8	98	25.5	98	26.5	98	29.0	99	30.9	99
400	22.8	97	24.9	98	25.4	97	26.0	96	28.9	99	30.9	99
1600	18.3	77	20.0	79	20.6	79	21.2	78	23.9	82	26.0	83

From Kuehborth et al. (1988a)

Table 38. Body weight after 12 and 24 months in rats administered cycloxydim sodium salt in the drinking-water

Concentration in drinking-water (ppm)	Body weight at 12 months				Body weight at 24 months			
	Males		Females		Males		Females	
	g	% of control	g	% of control	g	% of control	g	% of control
0	725.1	—	410.3	—	836.5	—	495.4	—
100	713.6	98	402.1	98	809.2	97	511.1	103
400	692.3	95	373.6**	91	828.9	99	457.4	92
1600	645.9**	89	339.3**	83	771.3	92	405.5**	82

From Kuehborth et al. (1988a)
** $P < 0.01$ (ANOVA + Dunnett's test)

There were no haematological changes that could be related to the administration of the test substance. Clinicochemical examinations revealed a reduction in triglyceride concentrations in females of the 1600 ppm and 400 ppm groups. Statistical significance was not attained owing to the high standard deviation in the control group. In the second determination, the reduction in triglyceride concentrations was confirmed, although, again, statistical significance was not attained (Table 39).

Urinalysis did not reveal any test substance–related changes. Organ weight determinations revealed reduced absolute liver weights in the 1600 ppm females. Relative liver weights were significantly decreased in high dose group females when based on brain weight, which was similar between treatment groups, but relative liver weights were increased in the same group when related to body weight, indicating that the absolute liver weight decrease was a result of the overall reduction in body weight rather than an organ-specific effect (Table 40).

Gross pathological and histopathological examinations did not show test substance–related effects at any dose level. In the liver, however, bile duct proliferation was more frequently recorded in treated males than in controls. The incidence across the groups 0 (control), 100, 400 and 1600 ppm

Table 39. Triglyceride concentrations at 24 months in rats administered cycloxydim sodium salt in the drinking-water

Concentration in drinking-water (ppm)	Triglyceride concentration			
	Males		Females	
	mmol/l	% of control	mmol/l	% of control
0	4.39	—	11.17	—
100	4.82	110	13.73	123
400	7.59	173	9.51	85
1600	6.79	155	5.24	47

From Kuehborth et al. (1988a)
Statistical evaluation: ANOVA + Dunnett's test

Table 40. Absolute and relative liver weights in rats administered cycloxydim sodium salt in the drinking-water for 2 years

Concentration in drinking-water (ppm)	Body weight		Absolute liver weight		Liver weight relative to body weight		Liver weight relative to brain weight	
	g	% of control	g	% of control	%	% of control	%	% of control
Males								
0	836.5	—	22.33	—	2.67	—	1028	—
100	809.2	97	21.37	96	2.64	99	986	96
400	828.9	99	21.08	94	2.54	95	991	96
1600	771.3	92	21.90	98	2.84	106	1020	99
Females								
0	495.4	—	15.31	—	3.09	—	754	—
100	511.1	103	15.52	101	3.04	98	757	100
400	457.4	92	14.07	92	3.08	100	712	94
1600	405.5**	82	13.52**	88	3.33	108	673*	89

From Kuehborth et al. (1988a)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided); no statistics available for liver weight relative to body weight

was 2% (control), 8%, 16% and 24% in males and 8% (control), 12%, 4% and 12% in females. The number of males with bile duct proliferation exhibited a statistically significant positive trend with respect to dose rate ($Z = 4.04$, one-tailed $P < 0.001$), but the maximum incidence of bile duct proliferation in the high dose group males of the 24-month study (24%) was similar to the control group incidence in the 18-month study (20%) and below the average range of historical control data for this age-associated lesion. In females, no statistically positive trend was evident. In addition, foci or areas of various types of hepatocellular alteration were more frequently recorded in males than in females. The relative proportion of males with basophilic foci was 7% (control), 22%, 28% and 28%. The frequency of the basophilic foci did not show a dose–response relationship. Both bile duct proliferation and basophilic foci in male rats exhibited a statistically significant positive trend with respect to dose rate, but the incidences were within the historical control range (Table 41).

Cycloxydim did not have carcinogenic properties.

The administration of cycloxydim via the drinking-water resulted in a reduction in body weight at concentrations of 400 ppm and 1600 ppm. Drinking-water consumption was reduced in the 1600 ppm group. In females, there was a reduction in triglyceride levels at concentrations of 400 and 1600 ppm. The NOAEL was 100 ppm, equal to 7 mg/kg bw per day, on the basis of a reduction in

Table 41. Histopathological changes in rats administered cycloxydim sodium salt in the drinking-water for 2 years

Concentration in drinking-water (ppm)	Incidence[a]				Historical data: bile duct proliferation		Historical data: basophilic foci[b]	
	Bile duct proliferation		Basophilic foci					
	18 months	24 months	18 months	24 months				
Males								
0	4/20 (20)	2/100 (2)	2/20 (10)	7/100 (7)	Time frame	1983–1992	Time frame	1983– 1992
100	4/20 (20)	4/50 (8)	1/20 (5)	11/50 (22)	Average	27%	Average	13.9%
400	3/20 (15)	8/50 (16)	7/20 (35)	14/50 (28)	Minimum	0%	Minimum	0%
1600	7/20 (35)	12/50 (24)	2/20 (10)	14/50 (28)	Maximum	65%	Maximum	65%
2700	8/20 (40)	—	2/20 (10)	—	Studies	21	Studies	21
Females								
0	0/20 (0)	8/100 (8)	5/20 (25)	8/100 (8)	Time frame	1983–1992	Time frame	1983–1992
100	1/20 (5)	6/50 (12)	0/20 (0)	5/50 (10)	Average	11%	Average	17%
400	1/20 (5)	2/50 (4)	3/20 (15)	8/50 (16)	Minimum	0%	Minimum	0%
1600	2/20 (10)	6/50 (12)	1/20 (5)	3/50 (6)	Maximum	75%	Maximum	56%
2700	0/20 (0)	—	0/20 (0)	—	Studies	21	Studies	21

From Kuehborth et al. (1988a)

[a] No. of occurrences/no. of animals examined; percentage given in parentheses.

[b] In early studies at the test facility, different liver foci types (i.e. eosinophilic, basophilic, clear cell, mixed cell) were subsumed under the diagnostic term "foci of hepatocellular alteration". No differential diagnosis for different foci types was made; therefore, foci subsets, including basophilic foci, were not listed in early studies. This is considered to be the reason for the apparent time-dependent shift in control group incidences (zero incidences in early studies, e.g. 1981–1983, and moderate to high incidences in later studies, e.g. 1988–1993), which is the consequence of the gradual introduction of foci type–specific quantification of hepatocellular foci by the test facility pathologists in the mid to late 1980s.

body weight and a reduction in concentrations of triglycerides in rats given drinking-water containing cycloxydim at concentrations of 400 ppm and above. Cycloxydim was not carcinogenic to rats. The study complied with GLP, and a QA statement was attached (Kuehborth et al., 1988a).

2.4 Genotoxicity

Cycloxydim was tested for genotoxicity in an adequate range of assays of 11 genotoxicity studies, of which 9 are in vitro studies and 2 are in vivo studies (Table 42). Cycloxydim acid and the sodium salt gave negative results throughout, except at cytotoxic concentrations in studies of chromosomal aberrations in vitro. Most of the studies complied with GLP, and QA statements were attached.

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

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Cycloxydim (purity 93.9%; batch No. N 88) was administered to groups of 24 male and 24 female Wistar rats (F_0 parental generation) as the sodium salt via the drinking-water at concentrations of 0, 100, 400 and 1600 ppm. At the beginning of the substance administration, the age of F_0 generation rats was 55 days, and the mean weights were 246.6 g and 168.0 g for males and females, respectively.

Table 42. Results of genotoxicity studies with cycloxydim

Test system	Test compound Strain/species	Concentration	Purity (%)	Result	Reference
In vitro					
Gene mutation Bacterial cells Ames test	Cycloxydim sodium salt *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 Standard plate technique	0, 20, 100, 500, 2000, 5000 µg/plate (1st experiment) 0, 60, 300, 1500, 7500, 15 000 µg/plate (2nd experiment)	93.9	Negative with S9 mix Negative without S9 mix	Engelhardt & Gelbke (1985b)
Gene mutation Bacterial cells Ames test	Cycloxydim (acid) *S. typhimurium* TA98, TA100, TA1535, TA1537 Standard plate technique	0, 20, 100, 500, 2500, 5000 µg/plate	92.2	Negative with S9 mix Negative without S9 mix	Engelhardt & Gelbke (1983)
Gene mutation Mammalian cells	Cycloxydim sodium salt CHO cells (*HPRT* locus)	5, 10, 15, 21, 28, 35, 40 mg/ml	93.9	Negative with S9 mix Negative without S9 mix	den Boer & Hoorn (1985)
Gene mutation Mammalian cells	Cycloxydim sodium salt CHO cells (*HPRT* locus)	Ranged from 0.215 to 21.5 mg/ml (1st experiment), 0.1 to 4.64 mg/ml (2nd experiment) and 0.215 to 4.64 mg/ml (3rd experiment)	93.9	Negative with S9 mix Negative without S9 mix	Jaeckh & Gelbke (1986)
Gene mutation/ chromosomal aberration Mammalian cells	Cycloxydim sodium salt Mouse lymphoma forward mutation assay L5178Y Tk+/− cells (*TK* locus)	Ranged from 1.75 to 20 mg/ml with and without metabolic activation (1st experiment) 5–12.5 mg/ml in the 2nd experiment (with metabolic activation only)	93.9	Weakly positive in the presence of cytotoxicity with S9 mix Negative without S9 mix	den Boer (1985)
Chromosomal aberration Mammalian cells	Cycloxydim sodium salt CHO cells	2000, 3333, 4000, 5000 µg/ml	93.9	Negative with S9 mix Weakly positive in the presence of cytotoxicity without S9 mix	Taalman (1985a)
Chromosomal aberration Mammalian cells	Cycloxydim (acid) CHO cells	500, 1666.6, 5000 µg/ml 16.6, 50, 166.6, 500, 1666.6 µg/ml	93.9	Negative with S9 mix Weakly positive in the presence of cytotoxicity without S9 mix	Taalman (1985b)
Unscheduled DNA synthesis	Cycloxydim sodium salt Rat primary hepatocytes	0.906, 1.81, 3.63, 9.06, 18.1, 36.3, 90.6 µg/ml	93.9	Negative	Cifone & Brusick (1985)
Unscheduled DNA synthesis	Cycloxydim (acid) Rat primary hepatocytes	100, 250, 375, 500, 750, 1000, 1500, 2000 µg/ml	93.9	Negative	Cifone & Myhr (1985)
In vivo					
Micronucleus test	Cycloxydim sodium salt NMRI mice	Oral (gavage, one application): 0, 225, 450, 900 mg/kg bw	93.9	Negative	Engelhardt & Gelbke (1985a)
Chromosomal aberration test	Cycloxydim sodium salt Chinese hamster	Oral (gavage, one application): 0, 500, 1700, 5000 mg/kg bw	36.56	Negative	Taalman (1987)

CHO, Chinese hamster ovary; S9, 9000 × *g* rat liver supernatant

A stock solution of the test substance was prepared by dissolving cycloxydim in 2 N NaOH. The end concentrations were achieved by dilution of the stock solution with drinking-water. The pH of the drinking-water solutions was adjusted to a neutral range (at a pH of between 7 and 8.3) with 0.5 N HCl solution.

The doses were selected based on the results of a 4-week study and a 3-month study and the preliminary results of a chronic study in the same rat strain and using the same route of administration (drinking-water studies with the sodium salt). At least 70 days after the beginning of treatment, one female was mated with one male overnight for a maximum period of 3 weeks to produce the F_1 litter. The F_0 generation was remated at least 10 days after the last weaning to produce a second litter. From the F_{1a} pups, 24 males and 24 females per dose group were selected as the F_1 parent generation to produce the F_2 generation (one litter). The F_1 generation parental animals received the test substance at least 98 days before mating (Table 43).

The examination of parental animals included monitoring for clinical symptoms/mortalities, food consumption, body weight development, mating and reproductive performance. Pathological examination was performed by gross inspection as well as extensive histopathological examination, with special attention to the organs of the reproductive system. Pups were sexed and monitored with respect to their developmental stages and behaviour in certain tests. All pups were examined macroscopically at necropsy (external and organ findings); stillborn pups and pups that died intercurrently were additionally examined for any skeletal findings.

Sufficient stability of the test substance was demonstrated for the duration of the study (93.9% at the start and 90.1% at the last determination), and its homogeneity was proven. The stability of the aqueous sodium salt solution as well as the correctness of intended concentrations (dose levels) were confirmed by analysis. The test substance intake is given in Table 44.

There were no clinical signs or mortalities at any dose level in parental animals treated with cycloxydim sodium salt that could be attributed to the test compound. Effects on food consumption, water consumption and body weight development are summarized in Table 45.

At the high dose level of 1600 ppm, the pattern of effects observed during the study differed between the F_0 and F_1 parents:

Table 43. Study design: two-generation drinking-water study in Wistar rats

Parameter	Concentration in drinking-water (ppm)			
	0 (control)	100	400	1600
F_0 generation – parent animals	Treatment period at day 55 postpartum for at least 70 days prior to mating to produce F_{1a} and F_{1b} pups until sacrifice. Infertile animals were remated with a fertile partner.			
- No. of males	24	24	24	24
- No. of females	24	24	24	24
F_1 generation – parent animals	Treatment at least 98 days prior to mating to produce F_2 pups (only one litter) until sacrifice. Infertile animals were remated with a fertile partner.			
- No. of males	24	24	24	24
- No. of females	24	24	24	24
$F_{1a/b}$ and F_{2a} generation pups	Reared until day 4 postpartum, when four males and four females were selected for further rearing until day 21 postpartum. The remainder were sacrificed and examined macroscopically (external and organs). Stillborn pups or pups that died as well as pups showing remarkable findings during rearing or abnormalities during macroscopic inspection were examined according to the method of Wilson (head) and Kimmel (modified) or X-rayed to examine the skeleton.			

From Hellwig et al. (1988)

Table 44. Test substance intake in a two-generation drinking-water study in Wistar rats

Parameter	Test substance intake (mg/kg bw per day)		
	Concentration in drinking-water (ppm)		
	100	400	1600
F_0 generation animals			
Premating period (days 0–70), males	7.46	30.66	113.26
Premating period (days 0–70), females	9.81	38.12	128.65
Average intake males/females	8.64	34.39	120.96
F_{1a} litter gestation period (days 0–20), females	10.59	43.37	149.09
F_{1a} litter lactation period (days 0–21), females	21.23	85.31	253.15
F_{1b} litter gestation period (days 0–20), females	10.19	37.85	120.82
F_{1b} litter lactation period (days 0–21), females	23.93	83.99	242.99
F_1 generation animals			
Premating period (days 0–98), males	9.68	38.19	132.18
Premating period (days 0–98), females	11.93	44.11	142.06
Average intake males/females	10.81	41.15	137.12
F_{2a} litter gestation period (days 0–20), females	10.36	42.38	149.90
F_{2a} litter lactation period (days 0–21), females	26.58	107.54	368.19

From Hellwig et al. (1988)

F_0 generation:

- reduced food consumption in females during gestation and lactation;
- reduced water consumption in females throughout the study, most pronounced during lactation of the F_{1a} and F_{1b} litters (65% and 56% of control value, respectively);
- reduced body weight and/or body weight gain in females, statistically significant during gestation and lactation.

F_1 generation:

- reduced food consumption in males and females during the premating period and in females during lactation;
- reduced water consumption in males during the premating period and in females throughout the study; more pronounced during the premating than during the lactation period (56% versus 77% of control value, respectively);
- reduced body weight and/or body weight gain in males and females, statistically significant decrease in females during lactation.

At the middle dose level of 400 ppm, these effects were less pronounced:

- water consumption was slightly reduced in F_0 dams during F_{1b} lactation (86%) and in F_1 females during F_{2a} premating (82% of control value);
- body weight and body weight gain were temporarily reduced in F_0 females during gestation and especially during lactation, whereas F_1 female body weight development appeared to have been marginally affected during the premating period.

Table 45. Effects on food consumption, water intake and body weight gain in a two-generation drinking-water study in Wistar rats

Parameter	Effects (% of control)								
	F_0 generation → F_{1a} litter			F_0 generation → F_{1b} litter			F_1 generation → F_{2a} litter		
	Concentration in drinking-water (ppm)								
	100	400	1600	100	400	1600	100	400	1600
Premating period (F_0: days 0–70; F_1: days 0–98)									
Food consumption[a]									
- Males	102	101	99	NA	NA	NA	98	98	87
- Females	100	98	96	NA	NA	NA	98	94	86
Water consumption[a]									
- Males	102	103	95	NA	NA	NA	99	96	71
- Females	103	100	81	NA	NA	NA	94	82	56
Body weight gain									
- Males	102	96	95	NA	NA	NA	96	142	85
- Females	98	96	85	NA	NA	NA	95	94	83
Gestation period (days 0–20 p.c.) females									
Food consumption[a]	96	93	90	95	91	87	96	96	93
Water consumption[a]	100	102	83	99	90	67	93	92	72
Body weight gain	96	91	77**	93	85**	73**	104	102	92
Lactation period (days 0–21 p.p.) females									
Food consumption[a]	86	90	76	90	87	71	99	100	86
Water consumption[a]	94	93	65	102	86	56	101	100	77
Body weight gain	76	62*	23**	106	91	78	127	146	111
Body weight (day 21 p.p.)	97*	95**	88**	98	95**	87**	100	99	88**

From Hellwig et al. (1988)
NA, not applicable; p.c., postcoitum; p.p., postpartum; * $P < 0.05$; ** $P < 0.01$ (Williams test)
[a] No statistical analysis performed.

No effects on food consumption, drinking-water uptake and body weight or body weight gain were noted at the low dose of 100 ppm that were considered treatment related.

There were no effects on reproductive function, such as mating performance, pregnancy rate, mean duration of pregnancy or littering/lactation of the offspring. The relatively low fertility index in the F_{1a} litter at 1600 ppm must be regarded as incidental, as all F_0 parents (sires and dams) proved to be fertile at the F_{1b} mating. In addition, no such findings were noted for the F_1 parental animals and the F_{2a} mating.

Treatment-related findings in pups (Table 46) were confined to the highest dose group of 1600 ppm. On the day of birth (day 0 postpartum [p.p.]), the mean number of live pups per litter was slightly reduced in all high dose group litters (F_{1a}, −6%, $P < 0.01$; F_{1b}, −10%, not statistically significant [n.s.]; and F_{2a}, −9%, n.s.). For the F_{1a} litters only, the reduction in the number of live pups per litter was accompanied by an increased mean number of dead pups per litter (0.84 versus 0.09, $P < 0.01$); however, the percentage of stillborn F_{1b} and F_{2a} pups of the high dose group was lower when compared with the respective control groups. The viability index appeared to be slightly reduced in the case of the high dose group F_{1b} pups, but was of a similar range in the high dose group F_{1a} and F_{2a} pups when compared with the control group indices. The lactation index was clearly decreased with regard to the F_{1a} and F_{1b} pups compared with the control, but not in the case of the F_{2a} pups, for which

Table 46. Pup findings in a two-generation study in Wistar rats (F_{1d}/F_{1b} and F_{2a} pups)

	Concentration in drinking-water (ppm)							
	0 (control)		100		400		1600	
No. of live pups per litter, day 0 p.p. (% of control)								
F_{1a} litter	12.65	(—)	12.26	(97)	11.70	(92)	11.89††	(94)
F_{1b} litter	14.74	(—)	14.29	(97)	13.54	(92)	13.22	(90)
F_{2a} litter	12.74	(—)	13.52	(106)	13.76	(108)	11.64	(91)
Viability index, day 4 versus day 0 p.p. (% of control)								
F_{1a} litter	95.94	(—)	88.75	(93)	94.98	(99)	90.58	(94)
F_{1b} litter	94.55	(—)	94.97	(100)	93.62	(99)	85.77	(91)
F_{2a} litter	94.10	(—)	94.33	(100)	94.12	(100)	92.77	(99)
Lactation index, day 21 versus day 4 p.p. (% of control)								
F_{1a} litter	87.63	(—)	86.83	(99)	87.51	(100)	70.74	(81)
F_{1b} litter	80.39	(—)	88.88	(111)	83.17	(103)	62.31†	(78)
F_{2a} litter	84.10	(—)	98.34	(117)	97.91	(116)	92.90	(110)
Pup body weight males, g (% of control)								
F_{1a} litter								
- Day 0	6.12	(—)	6.03	(99)	6.13	(100)	5.95	(97)
- Day 21	44.08	(—)	44.82	(102)	44.65	(101)	39.84	(90)
F_{1b} litter								
- Day 0	5.97	(—)	5.92	(99)	6.07	(102)	5.66**	(95)
- Day 21	41.09	(—)	39.73	(97)	38.58	(94)	33.85**	(82)
F_{2a} litter								
- Day 0	6.11	(—)	5.85	(96)	5.89	(96)	5.89	(96)
- Day 21	46.84	(—)	42.69	(91)	42.07*	(90)	41.65*	(89)
Pup body weight females, g (% of control)								
F_{1a} litter								
- Day 0	5.74	(—)	5.75	(100)	5.72	(100)	5.67	(99)
- Day 21	41.95	(—)	40.81	(97)	42.08	(100)	36.23*	(86)
F_{1b} litter								
- Day 0	5.67	(—)	5.58	(98)	5.71	(101)	5.30**	(93)
- Day 21	39.14	(—)	38.10	(97)	37.04	(95)	34.08**	(87)
F_{2a} litter								
- Day 0	5.77	(—)	5.63	(98)	5.56	(96)	5.64	(98)
- Day 21	46.21	(—)	41.11*	(89)	39.79*	(86)	41.86*	(91)
Litter size, day 21 p.p. (% of control)								
F_{1a} litter	10.65	(—)	10.00	(94)	9.91	(93)	8.16	(77)
F_{1b} litter	11.35	(—)	12.46	(110)	11.00	(97)	7.39	(65)
F_{2a} litter	10.39	(—)	12.87	(124)	12.95	(125)	10.09	(97)
Litter weight, day 21 p.p. (% of control)								
F_{1a} litter	469.5	(—)	448.2	(95)	442.5	(94)	325.1	(69)
F_{1b} litter	466.4	(—)	495.0	(106)	424.4	(91)	250.2	(54)
F_{2a} litter	483.3	(—)	539.3	(112)	530.0	(110)	421.3	(87)

From Hellwig et al. (1988)

* $P < 0.05$ / ** $P < 0.01$ (Williams trend test); † $P < 0.05$ / †† $P < 0.01$ (Krauth test)

the highest incidence of intercurrent deaths (i.e. lowest lactation index) was established in the control group. Pup weight and growth were reduced or retarded for all generations (F_{1a}, F_{1b} and F_{2a}).

A lower mean pup weight was also observed in F_{2a} pups at dose levels of 100 and 400 ppm. These reductions in mean pup weight should not be considered test substance related, but can be explained by the higher number of pups that died during rearing in the F_{2a} controls. Thus, dams of the 100 and 400 ppm test substance groups had to nurse more pups in comparison with the control group. The increased litter sizes at 100 and 400 ppm correspond to a 10% higher mean litter weight, however, causing a reduced weight development in the groups with high litter size.

In F_{1a} and F_{1b} pups of high dose group dams, morphological development appeared to be retarded: ear unfolding and opening were delayed in the F_{1b} litter, and eye opening was delayed in both F_{1a} and F_{1b} litters. In addition, an increased number of female F_{1a} pups failed in the gripping (holding) reflex test. These effects on morphological and behavioural development are considered to be related to the growth retardation noted for these pups.

There was no indication of any adverse effects on development in F_{2a} pups up to the highest dose tested.

With respect to external, internal and skeletal examination, there were no treatment-related effects on F_{1a}, F_{1b} or F_{2a} pups at any dose level.

In order to assess the effects on F_0 offspring during the lactation period, the quality and extent of maternal toxicity have to be taken into consideration. Besides decreased food consumption and resulting decreased body weight gain, the notably low consumption of drinking-water during gestation and lactation most probably resulted in decreased nutritional status of the F_{1a} and F_{1b} pups. The reduced water intake of the high dose group dams is considered to stem from the decreased palatability of the drinking-water in which the test substance was administered. Dams of the F_{1b} pups (in which the adverse effects observed were most remarkable) also showed the lowest water intake (56% of the control value). It is highly probable that less mothers' milk was available to pups of the high dose group F_0 dams compared with the other treatment group pups. A reduction in the milk quantity can be explained by the reduced water intake of dams (due to unfavourable palatability). This conclusion is substantiated by the fact that adverse effects of F_{2a} pups, whose dams consumed a considerably higher amount of drinking-water than did F_0 dams, were either non-existent or far less pronounced than in F_{1a} and F_{1b} pups. A direct effect of cycloxydim on milk quantity is highly unlikely. In conclusion, the growth retardation of high dose group pups during the lactation period is most probably related to a reduced water intake of the dams and not to a direct test substance–related effect on milk quality or quantity.

In view of the above findings and analysis, it can be concluded that cycloxydim administered as the sodium salt via the drinking-water to rats in a two-generation study caused clear signs of toxicity, such as reduced food consumption and depression of mean body weights and body weight gain in parental animals at the high dose level of 1600 ppm. In addition, a pronounced reduction in drinking-water consumption was observed at 1600 ppm. The number of live pups from high dose group F_{1a}, F_{1b} and F_{2a} litters was reduced at the day of birth, which, only in the case of the F_{1a} pups, was also associated with a slightly increased number of dead pups on day 0 p.p. Furthermore, the lactation index was decreased for F_{1a} and F_{1b} pups (not for F_{2a} pups) at 1600 ppm. High dose group pups from all generations showed reduced weight and retarded growth rate; morphological development (time of ear and eye opening) as well as gripping reflex test were impaired to some degree only in the case of F_1 pups. No adverse effects on morphological or behavioural development were seen in F_{2a} pups. Effects on high dose group F_{1a} and F_{1b} pups observed during the lactation period (increased mortality, delayed growth and development) were most probably a consequence of drastically reduced water intake by the F_0 dams (as low as 56% of the control level), which must have had an impact on milk quantity and hence resulted in malnutrition of their pups during lactation. The reduced water intake was considered to be related to unfavourable palatability of the drinking-water in which the test sub-

stance was offered to the dams. At 400 ppm, female parental animals were affected with respect to marginally reduced food and water consumption as well as reduced body weights and/or body weight gains during some parts of the study. No effects were noted for the pups at this dose level. A dose of 100 ppm did not show any findings at all. The fertility after administration of the test substance was not affected at any of the doses tested.

The NOAEL for offspring toxicity was 400 ppm, equal to 38 mg/kg bw per day, on the basis of reduced survival, reduced growth and developmental retardation in pups at 1600 ppm, equal to 129 mg/kg bw per day, the highest dose tested. Reproductive toxicity was not affected by treatment at drinking-water concentrations of up to 1600 ppm. The NOAEL for parental toxicity was 100 ppm, equal to 9.7 mg/kg bw per day, on the basis of reductions in food consumption, body weight and body weight gain in dams at 400 ppm. The study complied with GLP, and a QA statement was attached (Hellwig et al., 1988).

(b) Developmental toxicity

Rats

Cycloxydim (purity 93.9%; batch No. N 88) was prepared as a sodium salt and examined for its prenatal toxicity in Wistar rats. The dams (25 per test group) were treated from day 6 through day 15 postcoitum (p.c.) with daily cycloxydim doses of 0 (control treated with the vehicle, double-distilled water), 100, 200 and 400 mg/kg bw per day by gavage at a constant dosing volume of 10 ml/kg bw per day.

The animals were observed for food consumption and body weight gain regularly throughout the study period. Their state of health was checked daily. On day 20 p.c., all females were sacrificed and assessed by gross pathology. After caesarean section, reproductive parameters such as numbers of implantations, resorptions and corpora lutea were determined, and preimplantation and postimplantation loss and conception rate were calculated. The fetuses were dissected from the uterus, sexed and weighed. About two thirds of the fetuses were subjected to assessment of the skeleton; about one third of the fetuses were assessed for visceral abnormalities. The fetuses intended for assessment of the skeleton were also eviscerated, and the organs were assessed (after being fixed in ethanol).

Maternal data are compiled in Table 47. There were no clinical signs or mortalities noted in treatment group dams. Food consumption was slightly reduced at the onset of dosing (days 7 and 8), gaining statistical significance (about 8% absolute level) at the high dose level of 400 mg/kg bw per day and at the middle and high dose levels (4% and 8%, respectively) when compared on a milligram per kilogram body weight basis. At the end of the treatment period, the body weight gain at the high dose level was 13% below the control (days 13–15, no statistical significance). The assessment of the difference between the body weights of the dams at the end (without uterus) and at the beginning of the study showed a decrease of 9% at 400 mg/kg bw per day, which was statistically significant compared with control values. The slight, statistically significant decrease in food consumption at 200 mg/kg bw per day is considered incidental, because it did not correspond with changes in the net body weight change.

Evaluation of reproductive parameters following caesarean section (Table 48) revealed no treatment-related effects on uterus or placental weights, number of corpora lutea, incidence of live or dead implantations, incidence of early, intermediate or late resorptions, preimplantation or postimplantation loss, or incidence of live or dead fetuses. At the 400 mg/kg bw per day level, a statistically significant reduction in the mean fetal weight (especially in the male fetuses: −5.2%; overall: −4.3%) was observed. No significant effects on fetal weight were observed at 200 or 100 mg/kg bw per day.

The assessment of external abnormalities did not reveal any substance-induced increase compared with the control, even at the highest dose level. The incidence of skeletal changes was not increased up to a dose of 200 mg/kg bw per day. At a dose level of 400 mg/kg bw per day, however, a

Table 47. Maternal data: first prenatal toxicity gavage study in Wistar rats

	Effects on food consumption and body weight gain							
	Dose (mg/kg bw per day)							
	0		100		200		400	
	g	% of control	g	% of control	g	% of control	g	% of control
Food consumption								
- Absolute, days 7–8	47.6	—	48.0	101	45.8	96	43.8**	92
- Relative,[a] days 7–8	196.0	—	194.8	99	187.9*	96	179.8**	92
Body weight gain								
- Days 6–15	43.35	—	41.82	96	39.83	92	39.96[n.s.]	92
- Days 13–15	11.04	—	10.13	92	10.67	97	9.61[n.s.]	87
Corrected body weight gain[b]	65.1	—	69.2	106	63.1	97	59.5*	91

From Hellwig & Hildebrand (1987a)
* $P < 0.05$; ** $P < 0.01$ (Williams test); n.s., not statistically significant
[a] g/kg bw.
[b] Net weight change during the study.

Table 48. Mean fetal weight in first prenatal toxicity gavage study in Wistar rats

	Mean fetal weight							
	Dose (mg/kg bw per day)							
	0		100		200		400	
	g	% of control	g	% of control	g	% of control	g	% of control
Males	3.85	—	3.82	99.2	3.74	97.1	3.65**	94.8
Females	3.66	—	3.66	100	3.57	97.5	3.50*	95.6
Males and females	3.74	—	3.76	101	3.64	97.3	3.58*	95.7

From Hellwig & Hildebrand (1987a)
* $P < 0.05$; ** $P < 0.01$ (Williams test)

pronounced increase in changes of the vertebral column and the sternebrae was observed. Statistically significantly increased numbers of fetuses per litter with retardations of the skeleton were observed. Although these findings are also seen in the control, their increased incidence is clearly attributable to the test substance administration at 400 mg/kg bw per day and is in line with the reduced fetal body weight at this dose level. Statistically significant increases in the number of anomalies compared with the untreated control were found. The majority of anomalies were found mainly in the thoracic part of the vertebral column and consisted of dumbbell-shaped or bipartite vertebral bodies with involvement of the cartilage (Table 49).

Keeping in view the above facts, it can be concluded that cycloxydim when given as the sodium salt in aqueous solution from day 6 through day 15 p.c. to pregnant rats via gavage caused no effects on the maternal or fetal organism at 100 or 200 mg/kg bw per day. At the top dose of 400 mg/kg bw per day, maternal toxicity was evident with respect to a slight, statistically significant reduction in food consumption during onset of dosing (days 7–8). Body weight gain at the end of the treatment period (day 20) was 13% lower (no statistical significance) than control, as was the corrected body weight gain (9%; statistically significant). In fetuses, reduced fetal weight was observed at 400 mg/kg bw per day, which correlated with an increase in skeletal anomalies,

Table 49. Incidence of skeletal anomalies in fetuses in the prenatal rat study

Skeletal anomalies	Fetal incidence[a]			
	Dose level (mg/kg bw per day)			
	0	100	200	400
"Dumbbell"-shaped ossification centres, thoracic region	5/197 (2.5)	3/174 (4.6)	2/205 (4.4)	60/199 (30)
"Bipartite"-shaped ossification centres, thoracic region	0/197 (0.0)	4/174 (2.3)	2/205 (1.0)	7/199 (3.5)
"Bipartite" ossification centres of the xyphoid process	2/197 (1.0)	3/174 (1.7)	5/205 (2.4)	8/199 (4.0)
Insufficient cartilaginification of the 13th rib (ossification centres present)	21/197 (11)	15/174 (8.6)	23/205 (11)	33/199 (17)
"Bipartite" ossification centres of the vertebral bodies in the thoracic region (without "cones" of cartilage)	0/197 (0.0)	0/174 (0.0)	0/205 (0.0)	4/199 (2.0)
Incomplete ossification of the sternebrae (cartilage present)	10/197 (5.1)	14/174 (8.0)	31/205 (15)	66/199 (33)

From Hellwig & Hildebrand (1987a)

[a] No. of fetuses with anomalies/no. of fetuses examined; percentage given in parentheses.

both gaining statistical significance at this dose level. Skeletal anomalies were found mainly in the thoracic part of the vertebral column and consisted of dumbbell-shaped or bipartite vertebral bodies with involvement of the cartilage.

The NOAEL for maternal toxicity and embryo/fetotoxicity was 200 mg/kg bw per day. Increased numbers of fetuses per litter with retardations and a statistically significant increase in the frequency of anomalies of the vertebral column and the sternebrae with involvement of the cartilage and incomplete ossification were observed. The study complied with GLP, and a QA statement was attached (Hellwig & Hildebrand, 1987a).

A supplementary study in rats was undertaken to determine the dose of cycloxydim that elicits maternally toxic effects in pregnant animals. Investigation of the fetal toxicity was confined to assessment of resorption rates and to fetal weight determinations. Cycloxydim (purity 93.9%; batch No. N 88) as the sodium salt was administered to 25 Wistar rats per group from day 6 through day 15 p.c. with doses of 0 (control treated with the vehicle, double-distilled water), 200, 400, 600 and 800 mg/kg bw per day by gavage and at a constant dosing volume of 10 ml/kg bw per day. Half of the total daily dose was given twice a day at an interval of at least 4 h to avoid test substance flocculation and a change in pH, which would have occurred if 600 or 800 mg/kg bw per day would have been given in one dose daily.

The dams were observed for food consumption and body weight gain regularly throughout the study period. Their health was checked daily. Haematology (leukocytes, erythrocytes, haemoglobin, haematocrit, mean cell volume, mean haemoglobin content per erythrocyte, mean corpuscular haemoglobin concentration, platelets) and clinicochemical parameters (enzymes: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase; blood chemistry: sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, iron, copper, zinc, manganese) were determined on days 16 and 20 of the study in 10 animals to better understand maternal toxicity at these dose levels in pregnant rats. On day 20 p.c., all females were sacrificed and assessed by gross pathology. Reproductive parameters after caesarean section, such as numbers of implantations, resorptions and corpora lutea, were determined, and preimplantation and postimplantation loss and conception rate were calculated.

The fetuses were dissected from the uterus, sexed, weighed and examined externally. After fixation, they were examined macroscopically. No further fetal examinations were performed.

No mortalities were observed in any treatment group. One dam at 600 mg/kg bw per day temporarily showed reduced nutritional state, piloerection and intermittent respiration. At 800 mg/kg bw per day, two dams showed vaginal haemorrhages, and three further dams were found to have fur smeared with urine.

On days 7–8, there was a (substantially dose-dependent) reduction in food consumption in all of the treated groups. Whereas the marginal effect at the 200 mg/kg bw per day dose level was assessed as incidental in the absence of effects on body weight, the reduced feed intake at 400 mg/kg bw per day may have been test substance related, as it was associated with body weight loss at the same time point of investigation. The effect on food consumption at 600 and 800 mg/kg bw per day persisted throughout the treatment period. Compared with control values, a statistically significant decrease in body weight (mainly $P < 0.01$) was noted at the 600 and 800 mg/kg bw per day dose levels, whereas there was no statistical significance or biological relevance at 200 or 400 mg/kg bw per day throughout treatment. Body weight gain was affected on days 6–8 at 400, 600 and 800 mg/kg bw per day ($P < 0.01$). At later intervals, body weight gain was affected only in some rats at dose levels of 600 and 800 mg/kg bw per day, and overall body weight gain was reduced at 600 and 800 mg/kg bw per day (83.9% and 84.4%, respectively; $P < 0.01$). The corrected body weight gain was statistically significantly below the respective control value at 400 mg/kg bw per day ($P < 0.05$) and higher doses ($P < 0.01$).

Some clinicochemical parameters appeared to have been affected by treatment. The increase in creatinine values at the high dose level is in line with the toxicological profile of cycloxydim and has also been observed in other toxicological studies. It is unclear whether the increase in reticulocytes observed at the second examination point on day 20 should be interpreted as a compensatory effect of the bone marrow due to blood sampling at day 16 p.c. or as a consequence of the slightly reduced haemoglobin, erythrocyte and haematocrit values observed at the first sampling. The fact that other affected parameters could not be reproduced at the second sampling could be the consequence of the short time between both sampling points (4 days). Another possible explanation given by the study director is the fact that food consumption increased after substance administration had been stopped. Selected organ weights measured (absolute and relative liver, kidney and adrenal weights) showed no difference compared with the untreated control. Assessment of embryo/fetal mortality revealed no decrease in the number of live fetuses and no increase in prenatal mortality. Some macroscopic findings, such as absent/filiform tail, anal atresia and one skin defect, were observed in a very small number of fetuses at 600 mg/kg bw per day (two fetuses in two litters examined) or 800 mg/kg bw per day (four fetuses in three litters examined). Fetal weight was statistically significantly decreased at 600 and 800 mg/kg bw per day.

In view of the above, it is concluded that increased creatinine values at 800 mg/kg bw per day, decreased inorganic phosphate levels at 400–800 mg/kg bw per day and a weak effect on red blood cells (reduced haemoglobin, erythrocyte and haematocrit values) at doses of 400 mg/kg bw per day and above were indicative of maternal toxicity. Clinical findings (vaginal haemorrhages, fur smeared with urine) in a few animals at 800 mg/kg bw per day were also indicative of maternal toxicity.

A full-scale examination of the fetuses was not the scope of this study, and therefore fetuses were not examined for internal or skeletal changes. Nevertheless, there is no indication that the NOAEL for parameters examined is lower than that in the first study. Fetal weights were reduced at 600 and 800 mg/kg bw per day with statistical significance. The types of external findings at the 600 and 800 mg/kg bw per day dose levels (filiform tail with caudal vertebrae absent, anal atresia, dorsal skin defect, absent tail) have also been observed in the historical control.

The NOAEL for maternal toxicity was established at 200 mg/kg bw per day. A NOAEL for embryo/fetotoxicity was not derived owing to the limited scope of examinations performed. The study complied with GLP, and a QA statement was attached (Hellwig & Hildebrand, 1987c).

A second supplementary study in rats was undertaken to investigate the persistence of the changes to vertebral bodies observed in the prenatal toxicity study at high dose levels of cycloxydim (i.e. 600 and 800 mg/kg bw per day) observed in the above study. Cycloxydim (purity 93.9%; batch No. N 88) as the sodium salt was administered by gavage to pregnant rats at a dose level of 400 mg/kg bw per day from day 6 through day 15 p.c. The effects noted in dams for the prenatal segment were comparable with those noted in the previous prenatal toxicity study conducted in the same rat strain and described above (Hellwig & Hildebrand, 1987a). The maternal body weight gain was clearly reduced during the first days of treatment, and body weights were slightly reduced during the rest of gestation. Corrected body weight was decreased by 9%. The pattern of findings at caesarean section (decreased fetal weight) and skeletal findings (marked increase in dumbbell-shaped or bipartite vertebral bodies and retarded skeletal ossification) also corresponded to the results of the main prenatal toxicity study in rats. In the postnatal segment, the same pattern of maternal effects was noted. Perinatal mortality (deaths on days 0–1) was slightly increased in pups from dams sacrificed on day 21 p.p., but not in pups from dams killed on day 7 p.p., although treatment was identical. It is noteworthy that the incidence of specific skeletal "anomalies" is reduced by two thirds in a comparison of the total fetal incidence (day 20 p.c.) with the pup incidence approximately 3 weeks later (day 21 p.p.).

As this study was designed to compare the effect level for skeletal anomalies from the late fetal stage through pup development until day 21 p.p., no NOAEL determination has been taken into consideration for this study. The study complied with GLP, and a QA statement was attached (Hellwig & Hildebrand, 1987b).

Rabbits

This study was undertaken for ascertaining the prenatal toxicity of cycloxydim in Himalayan rabbits (strain Chhb:HM) in accordance with the USEPA/Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines, OECD test guideline 414 and GLP of USEPA/FIFRA (Merkle & Hildebrand, 1985). Subsequently, however, an amendment to the study was done. Instead of discarding the skeleton of rabbits after evaluation of X-ray pictures, the skeletons of all rabbit fetuses in the highest dose group (400 mg/kg bw per day) and individual fetuses in the control group were stained according to the method of Kimmel for comparison and assessed (Hellwig, 1986).

The sodium salt of cycloxydim (purity 93.9%; batch No. N 88) in aqueous medium was administered to 15 female Himalayan rabbits (strain Chhb:HM) per test group by gavage from days 6 through 18 postinsemination (p.i.). Doses selected were 0 (control), 100, 200 and 400 mg/kg bw per day in a constant volume of 10 ml/kg bw per day. The control group was administered the vehicle (distilled water) only. Body weights and food consumption were monitored throughout the study. The animals were examined twice daily for mortality and clinical symptoms. All surviving animals were sacrificed on day 29 p.i., and the fetuses were delivered by caesarean section. Postmortem examinations included gross macroscopic examinations of all internal organs. Reproductive parameters after caesarean section, such as uterine weight and numbers of corpora lutea, implantations, resorptions and live/dead fetuses, were determined. Fetuses were sexed, weighed and further examined for any external, soft tissue and skeletal findings. In addition to X-ray analysis, the fetuses from the 400 mg/kg bw per day dose groups and individual fetuses from the control group were stained according to the method of Kimmel and assessed, comparable to the developmental studies in rats (Hellwig et al., 1988), in order to determine whether the vertebral body effects observed in rats also occur in rabbits using similar examination techniques.

One dam from the low dose group and one dam from the high dose group died shortly before the 8th and 10th days of treatment, respectively. As both deaths occurred during handling prior to dosing of the test substance, an influence of the test substance can be ruled out. Clinical signs were confined to one case of premature birth at 200 mg/kg bw per day and one case of abortion by a doe dosed with 400 mg/kg bw per day. Findings of premature birth and abortion are common in this

Table 50. Findings at caesarean section in a developmental toxicity study in rabbits administered cycloxydim sodium salt

Parameter	Dose level (mg/kg bw per day)				Historical control data[a]			
	0	100	200	400	A	B	C	D
Uterine weights (g)	341.86	322.92	343.23	284.58*	ND	ND	ND	ND
No. of implantations per pregnant animal	7.21	7.31	6.92	6.75	4.94	5.57	6.31	5.47
% viable implantations per pregnant animal	90.94	87.50	87.67	81.53†	68.75	90.00	91.01	83.01
% resorptions per pregnant animal	9.06	12.50	12.33	18.47†	31.25	10.00	9.00	17.00
- No. of early resorptions	9	10	9	8	ND	ND	ND	ND
- No. of intermediate resorptions	0	0	0	4	ND	ND	ND	ND
- No. of late resorptions	1	2	2	2	ND	ND	ND	ND
- No. of dead fetuses	0	0	0	1	ND	ND	ND	ND
Mean fetal weight (g)	39.3	37.3	41.2	36.5	ND	ND	ND	ND

From Merkle & Hildebrand (1985)

ND, not determined; * $P < 0.05$ (Williams test); † $P < 0.05$ (Krauth asymptotic test)

[a] Historical control data compiled for Chbb:HM Himalayan rabbit; vehicles used: A, carboxymethyl cellulose; B, 0.5% NaCl percutaneously, C, inhalation; D: oil.

rabbit strain; as there was only one case of each in the 200 and 400 mg/kg bw per day groups, these findings were not considered substance related.

At 400 mg/kg bw per day, food consumption was statistically significantly reduced from day 7 to day 21 p.i. ($P < 0.01$, Williams test), especially towards the end of test substance administration during days 15–18 p.i., when food consumption was reduced by more than 56% compared with control feed intakes. Food consumption of high dose group rabbits increased after the end of the administration period and was even higher than in controls during days 22–29 p.i. During the administration period, the dams continuously lost weight, amounting to a total of 100 g from days 6 to 18 p.i. (−4% of the initial body weight), whereas control rabbits gained weight by about 69 g (+3% of the initial body weight) during gestation days 6–18. Statistically significant reductions in body weight were observed on days 16–21 p.i. ($P < 0.05$, Williams test); thereafter, high dose group rabbits gained body weight from days 21 to 29 p.i. (i.e. after the end of treatment). The mean net body weight gain ($[bw_{end}$ − uterine weight] − bw_{start}) at 400 mg/kg bw per day was −120 g compared with −68.5 g for control group dams; statistical significance was not attained for this difference.

At 200 mg/kg bw per day, mean daily feed intakes were transiently reduced during days 12–16 p.i. (77–80% of control intakes, statistically not significant) but were similar to control levels for the other time points of investigation. Body weight gain was reduced on days 11–14 p.i. (6.86 g versus 42.36 g; $P < 0.05$, Williams test) and increased on days 21–23 p.i. (36.14 versus 10.29 g; $P < 0.05$, Williams test). No other statistically significant effects on body weight or body weight gain were observed during the study. The corrected body weight gain was comparable to control levels.

At 100 mg/kg bw per day, there were no effects observed that were suggestive of maternal toxicity.

At the high dose level, uterine weights were decreased by 17% compared with control values. The decreased uterine weight was considered to be the consequence of a slight increase in the number of dead implantations in combination with a slight decrease in the number of viable fetuses. As can be seen from the historical control data, the percentage of resorptions varies to a great extent. As there was no meaningful difference concerning the individual stages of resorptions between the treated groups and the control group, this slight effect was considered to be of a spontaneous nature. No other parameters were affected at caesarean section (Table 50).

Table 51. Total incidence of anomalies in a development toxicity study in rabbits

Parameter	Incidence[a]				Historical control data[b]		
	Dose level (mg/kg bw per day)						
	0	100	200	400	Mean	Minimum (%)	Maximum (%)
Total anomalies							
- Fetal incidence	58/91(64)	67/83 (81)	70/79 (89)	62/66 (94)			
- Litter incidence	13/14 (92.86)	13/13 (100)	13/13 (100)	12/12 (100)			
- % affected fetuses per litter	*63.20*	*80.13*	*87.40**	*92.98***			
Fetal external anomalies ("caesarean section")							
- Fetal incidence	0/91 (0)	2/83 (2)	1/79 (1.3)	8/66 (12)	24/1552 (1.5)	0.0	5.3
- Litter incidence	0/14 (0.0)	2/13 (15.4)	1/13 (7.7)	4/12 (33.3)	18/247 (7.3)	0.0	15.4
- % affected fetuses per litter	*0.0*	*3.53*	*1.10*	*9.62*	1.4	0.0	4.1
Soft tissue ("organ") anomalies							
- Fetal incidence	58/91 (64)	65/83 (78)	69/79 (87)	57/66 (86)	434/1552 (28.0)	12.1	58.7
- Litter incidence	13/14 (92.9)	13/13 (100)	13/13 (100)	12/12 (100)	182/247 (73.7)	46.2	100
- % affected fetuses per litter	*63.20*	*77.75*	*86.30**	*85.24*	28.6	11.3	58.0
Fetal skeletal anomalies							
- Fetal incidence	3/91 (3)	4/83 (5)	1/79 (1.3)	10/66 (15)	181/1552 (11.7)	4.1	19.8
- Litter incidence	2/14 (14.3)	3/13 (23.1)	1/13 (7.69)	7/12 (58.3)	117/247 (47.4)	14.3	80.0
- % affected fetuses per litter	*3.06*	*4.33*	*0.96*	*16.79*	12.0	4.1	24.7

From Merkle & Hildebrand (1985)

* $P < 0.05$; ** $P < 0.01$ (Krauth test, asymptotic)

[a] No. of fetuses (litters) with anomalies/no. of fetuses (litters) examined; percentage is in parentheses.

[b] Historical control data: 20 prenatal toxicity studies with Himalayan rabbits (supplier: Dr. K. Thomae) of the test facility started between 1986 and 1993.

The total incidence of anomalies and the incidence of specific anomalies may be seen in Table 51 and Table 52, respectively. From these tables, it can be seen that an increased incidence of affected fetuses per litter was established at 200 and 400 mg/kg bw per day. The specific anomalies are separated origin of carotids (also termed carotid branching abnormality), pseudoankylosis, asymmetrical sternebrae and fused sternebrae.

At the maternally toxic dose of 400 mg/kg bw per day, the percentage of viable implantations per dam was significantly decreased. The percentage of resorptions and skeletal anomalies was also increased (still within the historical control range, but possibly treatment related). The incidence of pseudoankylosis (flexure of paw, a common external abnormality) was increased over controls and was outside the historical control range, suggesting a substance-induced effect, at 400 mg/kg bw per day. A statistically significantly increased incidence of total anomalies (on a percentage affected fetuses per litter basis, not on a litter basis) at 200 and 400 mg/kg bw per day was mainly due to an increased occurrence of the strain-specific soft tissue abnormality "separated origin of carotids". The reason for these higher incidences is unclear, but the anomalies are nevertheless likely to be of a spontaneous origin. Owing to the very frequent occurrence of this abnormality, the increased incidence in the treatment groups is considered to be marginal.

Table 52. Incidence of specific anomalies in a developmental toxicity study in rabbits

Anomalies	Incidence[a]				Historical control data[b]		
	Dose (mg/kg bw per day)						
	0	100	200	400	Mean	Minimum (%)	Maximum (%)
External anomalies detected at caesarean section							
Pseudoankylosis							
- Fetal incidence	0/91 (0)	2/83 (2.4)	1/79 (1.3)	8/66 (12)	24/1552 (1.5)	0.0	5.3
- Litter incidence	0/14 (0)	2/13 (15.4)	1/13 (7.7)	4/12 (33.3)	18/247 (7.3)	0.0	15.4
Meningocele							
- Fetal incidence	0/91 (0)	0/83 (0)	0/79 (0)	1/66 (1.5)			
- Litter incidence	0/14 (0)	0/13 (0)	0/13 (0)	1/12 (8.3)			
Scoliosis							
- Fetal incidence	0/91 (0)	0/83 (0)	0/79 (0)	1/66 (1.5)			
- Litter incidence	0/14 (0)	0/13 (0)	0/13 (0)	1/12 (8.3)			
Soft tissue anomalies							
Separated origin of the carotids							
- Fetal incidence	58/91 (64)	65/83 (78)	69/79 (87)	57/66 (86)	315/1552 (20.3)	5.3	58.7
- Litter incidence	13/14 (93)	13/13 (100)	13/13 (100)	12/12 (100)	145/247 (58.7)	15.4	93.3
Gallbladder agenesis							
- Fetal incidence	1/91 (1.1)	1/83 (1.2)	2/79 (2.53)	2/66 (3)	14/1552 (0.9)	0.0	4.7
- Litter incidence	1/14 (7.14)	1/13 (7.69)	2/13 (15.38)	1/12 (8.33)	10/247 (4)	0.0	15.4
Skeletal anomalies							
Asymmetrical sternebra(e)[c]							
- Fetal incidence	3/91 (3.3)	3/83 (3.6)	1/79 (1.3)	5/66 (7.6)	38/1552 (2.4)	0.0	8.1
- Litter incidence	2/14 (14.3)	2/13 (15.4)	1/13 (7.7)	4/12 (33)	35/247 (14.2)	0.0	38.5
Fused sternebra(e)							
- Fetal incidence	0/91 (0)	0/83 (0)	0/79 (0)	4/66 (6.1)	66/1552 (4.3)	0.0	8.8
- Litter incidence	0/14 (0)	0/13 (0)	0/13 (0)	3/12 (25)	46/247 (18.6)	0.0	38.5

From Merkle & Hildebrand (1985)

[a] No. of fetuses (litters) with effect/no. of fetuses (litters) examined; percentage given in parentheses.

[b] Historical control data: 20 prenatal toxicity studies with Himalayan rabbits (supplier: Dr. K. Thomae) of the test facility started between 1986 and 1993.

[c] Asymmetrical sternebrae are now summarized under the term "sternebra(e) with irregular shape".

The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on transiently reduced mean daily food intakes during days 12–16 p.i. and reduced body weight gain on days 11–14 p.i. The maternal toxicity observed at doses of 200 and 400 mg/kg bw per day occurred late in the study, indicating that repeated dosing over several days was required to elicit the effect. At 400 mg/kg bw per day, the percentage of viable implantations per dam was decreased, and the incidence of several skeletal anomalies (e.g. asymmetrical sternebrae and fused sternebrae) was increased above the range for the historical controls. The NOAEL for embryo/fetotoxicity was 200 mg/kg bw per day. The study complied with GLP, and a QA statement was attached (Merkle & Hildebrand, 1985).

(c) In vitro studies

Rats

An in vitro study was performed to further elucidate if the anomalies on the skeleton observed in the in vivo rat studies at a dose level of 400 mg/kg bw per day could also be induced without contribution of the maternal organism and to exclude maternal toxic effects. For this purpose, rat embryos were exposed directly to cycloxydim at a concentration of 300 µg/ml and to the main metabolite BH 517-TSO (purity not reported; batch No. L28/145) at a concentration of 150 µg/ml. Based on evaluation of toxicokinetic data, the chosen cycloxydim concentration was considered to correspond to peak serum levels that result from oral gavage treatment at 400 mg/kg bw per day (oral dose at which the skeletal changes of the thoracic vertebra were observed). For the main metabolite, the peak serum concentration was assumed to be less than half of the cycloxydim peak serum level.

The rat embryos aged 9.5 days were exposed for 48 h to the culture medium, with addition of dimethyl sulfoxide (vehicle control was used to dissolve the test compounds), cycloxydim or the BH 517-TSO metabolite. At least 30 embryos were used for the three subexperiments carried out on each tested compound. The following parameters were examined: yolk sac diameter, crown–rump length, number of somite pairs, protein content (micrograms per embryo), developmental score according to Klug and embryos showing abnormal development (macroscopic and histological evaluation).

Following 48 h of in vitro exposure of fetuses to BH 517-TSO at 150 µg/ml, no evidence of abnormal development was detectable using various parameters, including histology. Fetal exposure to cycloxydim at 300 µg/ml resulted in a slight, statistically significant growth retardation (evident as reduced protein content and a very slightly reduced crown–rump length that led to a very slightly reduced overall score). These changes can be interpreted as unspecific signs of cytotoxicity. It is noteworthy that such alterations are also observed with any test compound if the concentration is sufficiently high. However, no abnormal morphogenetic differentiation could be observed when the embryos were examined with a stereomicroscope. The extensive histological investigations also revealed no evidence for a substance-related interference with morphogenetic differentiation. Some necrosis observed was within the physiological range of developing embryonic tissues.

In view of the above, it can be concluded that no abnormal morphogenetic differentiation was observed in vitro using whole embryo culture technique in 9.5-day-old Wistar rat embryos when they were exposed to concentrations of cycloxydim or the BH 517-TSO metabolite at 300 µg/ml and 150 µg/ml, respectively. These serum concentrations are expected after oral dosing of 400 mg/kg bw per day of cycloxydim based on toxicokinetic data. In particular, no signs of abnormal development could be noted at the developing somites that will give rise to the vertebrae. A slight growth retardation of embryos exposed to cycloxydim was considered to be an expression of unspecific cytotoxicity. The study was not undertaken according to any approved guideline or GLP (Neubert, 1987b).

2.6 Special studies

(a) Neurotoxicity studies

Studies on the potential neurotoxicity of cycloxydim have not been carried out. However, there were no clinical signs that would suggest a neurotoxic potential in any of the species tested, even at very high dose levels. In addition, standard histopathological examinations of neuronal tissue gave no indication of a test substance–related effect.

(b) Studies on metabolites/impurities

Different studies have been undertaken for four compounds that are either present as impurities in technical cycloxydim or present as plant metabolites and do not figure in animal metabolism. These are:

Table 53. In vitro genotoxicity assays with BH 517-TSO, a cycloxydim metabolite/impurity

End-point	Test object	Concentration	Purity (%)	Result	Reference
Salmonella typhimurium/ *Escherichia coli* reverse mutation assay	Ames test (standard plate and preincubation test) *S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA	0 (solvent control), 22.5, 111, 555, 2775 and 5500 µg/plate (0.1 ml of each concentration per plate)	90.8	Negative (with and without metabolic activation)	Engelhardt & Leibold (2004)[a]

[a] Complied with GLP.

Table 54. Acute toxicity of BH 517-5-OH-TSO, a cycloxydim metabolite

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Effects	Reference
Rat	Wistar	Male and female	Oral	>2000	No clinical findings	Gamer & Hoffmann (2001)[a]

[a] Complied with GLP.

- *BH 517-TSO*—This has been identified as an impurity in technical cycloxydim batches and as a metabolite in plants, animals, soil and water.
- *BH 517-5-OH-TSO*—The relevant residues in plant agricultural commodities include the 5-hydroxylated metabolite species, which has not been detected in animal matrices in appreciable amounts.
- *BH 517-TGSO*—This cycloxydim metabolite is a substituted glutaric acid derivative of cycloxydim and has been identified as a plant metabolite in straw of soybean and maize, in maize forage and, at a very low level, in sugar beet roots.
- *BH 517-TGSO*$_2$—This cycloxydim metabolite is a substituted glutaric acid derivative of cycloxydim and has been identified as a plant metabolite in straw of soybean and maize, in maize forage and, at a very low level, in sugar beet roots.

BH 517-TSO

BH 517-TSO was tested for genotoxicity in vitro, with negative results (Table 53).

BH 517-5-OH-TSO

BH 517-5-OH-TSO is of low acute oral toxicity in rats; no mortality or clinical symptoms were observed at the limit dose of 2000 mg/kg bw (Table 54).

Cycloxydim metabolite BH 517-5-OH-TSO (purity 90.3%; batch No. 01743-66) was tested in a 3-month dietary study in rats. This study was undertaken in order to establish a NO(A)EL, as this metabolite to which humans could potentially be exposed has not been identified in the relevant toxicity studies with the active substance. According to the European Commission draft guidance document on relevant metabolites, "A NOAEL of 50 mg/kg body weight per day is considered sufficient for a hazard approach". This principle was adopted in this study. Thus, BH 517-5-OH-TSO was administered via the diet to 10 male and 10 female Wistar rats per group at dose levels of 0 and 50 mg/kg bw per day for 3 months. The animals were observed daily for clinical symptoms and mortalities. An FOB and measurement of motor activity were carried out towards the end of the observation period. Ophthalmological examinations were carried out prior to the start and towards the end of the administration period. Body weight and food consumption were measured weekly. Clinicochemical and haematological examinations as well as urinalysis were performed on all animals at the end of the administration period. All animals were subjected to a full macroscopic and histopathological examination. Weights of 11 selected organs were determined.

Table 55. In vitro genotoxicity of BH 517-5-OH-TSO, a cycloxydim metabolite

End-point	Test object	Concentration	Purity (%)	Result	References
Salmonella typhimurium/ *Escherichia coli* reverse mutation assay	Ames test (standard plate and preincubation test) *S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA	22.2–5550 µg/plate (1st experiment) 20–5000 µg/plate (2nd experiment)	90.3	Negative (with and without metabolic activation)	Engelhardt & Hoffmann (2000)[a]
In vitro gene mutation test	Chinese hamster ovary (CHO-K1) cells	225–3600 µg/ml	90.3	Negative (with and without metabolic activation)	Engelhardt & Hoffmann (2001a)[a]
In vitro chromosomal aberration assay	V79 cells	1st experiment 0, 900, 1800 and 3600 µg/ml (4 h exposure time, 18 h harvest time, with and without S9 mix) 2nd experiment (for confirmation) 0, 900, 1800 and 3600 µg/ml (18 h exposure time and 28 h harvest time, without S9 mix) 0 and 3600 µg/ml (18 h exposure time and 28 h harvest time, without S9 mix) 0, 900, 1800 and 3600 µg/ml (4 h exposure time, 28 h harvest time, with S9 mix)	90.3	Negative (with and without metabolic activation)	Engelhardt & Hoffmann (2001b)[a]

[a] Complied with GLP.

Table 56. In vitro genotoxicity assays with BH 517-TGSO, a cycloxydim metabolite

End-point	Test object	Concentration	Purity (%)	Result	Reference
Salmonella typhimurium/ *Escherichia coli* reverse mutation assay	Ames test (standard plate and preincubation test) *S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA	21.2, 106, 530, 2650 and 5300 µg/plate in standard plate test, and 331.3, 662.5, 1325, 2650 and 5300 µg/plate in preincubation assay	94.0	Negative (with and without metabolic activation)	Schulz & Landsiedel (2008a)[a]

[a] Complied with GLP.

As no abnormalities were found in the parameters examined, the NOAEL of BH 517-5-OH-TSO in this study is 50 mg/kg bw per day (i.e. the highest dose tested). The study complied with GLP, and a QA statement was attached (Mellert et al., 2001b).

BH 517-5-OH-TSO was tested for genotoxicity in vitro, with negative results (Table 55).

BH 517-TGSO

BH 517-TGSO was tested for genotoxicity in vitro, with negative results (Table 56).

BH 517-$TGSO_2$

BH 517-$TGSO_2$ (Reg. No. 356102; purity 80%; lot/batch No. TP08/152/1/1) was administered to groups of five male and five female Wistar (Crl:WI(Han)) rats in the drinking-water at concentrations of 0, 1000, 3000 and 6000 ppm (equal to 0, 81.6, 240.4 and 440.5 mg/kg bw per day for males

Table 57. In vitro genotoxicity assays with BH 517-TGSO$_2$, a cycloxydim metabolite

End-point	Test object	Concentration	Purity (%)	Result	References
Salmonella typhimurium/ *Escherichia coli* reverse mutation assay	Ames test (standard plate and preincubation test) *S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA	0.4–5000 µg/plate in standard plate test and 0.4–2500 µg/plate in preincubation assay	80.0	Negative (with and without metabolic activation)	Schulz & Landsiedel (2008c)[a]
In vitro forward mutation assay in mammalian cells (HPRT test)	CHO-K1 cells	168.8–3300 µg/ml	80.0	Negative (with and without metabolic activation)	Schulz & Landsiedel (2008b)[a]
In vitro chromosomal aberration assay in mammalian cells	V79 cells	Up to 3300 µg/ml (10 mmol/l)	80.0	Negative (with and without metabolic activation)	Schulz & Landsiedel (2008d)[a]

[a] Complied with GLP.

and 0, 88.7, 293.1 and 560.9 mg/kg bw per day for females, respectively) for a period of 4 weeks. The rats were 42 ± 1 days old at the start of administration, and body weights at dosing were 184.8–188.2 g (male rats) and 145.9–154.3 g (female rats). Food consumption, water consumption and body weights were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. An FOB and measurement of motor activity were carried out after about 4 weeks of treatment in males and females. Clinicochemical and haematological examinations as well as urinalyses were performed towards the end of the administration period. All animals were assessed by gross pathology, followed by histopathological examinations.

The oral administration of BH 517-TGSO$_2$ to Wistar rats in drinking-water over a period of 4 weeks did not cause any signs of toxicity. Therefore, the NOAEL for BH 517-TGSO$_2$ under the conditions of the present study was 6000 ppm, equal to 440.5 mg/kg bw per day in males and 560.9 mg/kg bw per day in females. The study complied with GLP, and a QA statement was attached (Kaspers et al., 2008).

BH 517-TGSO$_2$ was tested for genotoxicity in vitro, with negative results (Table 57).

3. Observations in humans

No reports of adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to cycloxydim were available, except for three cases of eye irritation that occurred during production/filling of an old formulation, “Focus Ultra”.

3.1 Medical surveillance on manufacturing plant personnel

The BASF personnel who were handling cycloxydim in formulation were surveyed by regular medical examinations. This surveillance programme was not aimed specifically at detecting cycloxydim-related symptoms or diseases. Thus, it was not possible to evaluate a causal association between the compound and any specific medical effect.

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

No poisoning incidents were reported. Three cases of eye irritation occurred during the production/filling of "Focus Ultra" (BAS 517 22 H), on 7 February 1997, 9 October 1998 and 15 June 2001. Meanwhile, BAS 517 22 H has been replaced by the new Focus Ultra formulation "BAS 517 24 H", for which there is no evidence of eye irritation from animal studies or from case reports.

However, no observations regarding health effects after exposure of the general public are known to the manufacturer.

4. Literature review

A search in literature databases for published toxicological data on cycloxydim was performed on 15 June 2009 via the online database platform STN (Karlsruhe), which included CAS No. and synonyms of cycloxydim as search parameters. Databases searched via STN were AGRICOLA, ESBIOBASE, HEALSAFE, TOXCENTER, CABA, EMBAL, LIFESCI, CSNB, EMBASE and NTIS.

Based on the results of the literature search, only one publication of potential relevance was identified and therefore retrieved for a detailed review.

In order to establish in vitro tools to study mammalian acetyl coenzyme A (CoA) carboxylase (ACC) activity, Seng et al. (2003) investigated the ability of different members of the cyclohexanedione class of herbicides, including cycloxydim, to inhibit ACC. ACC plays an important role in lipid metabolism. For this reason, ACC came into the focus for therapeutic intervention in metabolic diseases. The monocot chloroplast enzyme is the selective target of cyclohexanedione herbicides. In contrast to other publications, where it is known that cyclohexanedione does not inhibit mammalian ACC activity, Seng et al. (2003) reported a competitive inhibition of rat heart ACC activity. Seng et al. (2003) measured ACC activity from homogenized rat hearts in vitro by the carboxylation of acetyl CoA to form malonyl CoA. Beside cycloxydim, the cyclohexanedione herbicides alloxydim, clethodim, sethoxydim, trepraloxydim and tralkoxydim were tested. With the exception of alloxydim, all investigated cyclohexanedione herbicides were found to inhibit ACC with similar potency (median inhibitory concentration [IC_{50}] values ranging between 18 and 40 nmol/l); alloxydim was much less potent. The results were plotted poorly, and the IC_{50} values for each tested cyclohexanedione cannot be read from the graph. The IC_{50} values were given only as a summarized range for all tested cyclohexanediones and not in detail. Thus, the poor presentation of the results does not allow any firm conclusions to be drawn (Seng et al., 2003).

Comments

Biochemical aspects

Both the free acid and the sodium salt of cycloxydim are well absorbed; bioavailability was approximately 100%. The results of excretion balance studies indicated that most (74–86%) of a single oral dose of the sodium salt of cycloxydim at 10 mg/kg bw per day is eliminated via the urine, most being excreted within 24 h. Biliary excretion (50–65% of the administered dose) and enterohepatic circulation play an important role in the elimination of cycloxydim. The highest concentrations of radiolabel were found in the liver and the kidneys. Quantities of radiolabel in all organs rapidly declined over time. There was no evidence for bioaccumulation of cycloxydim. The pattern of metabolites in the urine was similar for the free acid and the sodium salt of cycloxydim, and AUC data indicated that elimination was saturable at higher doses. The major metabolite in the urine and bile was the sulfoxide of cycloxydim, BH 517-TSO. Additional metabolites identified were BH 517-

T1SO (derived from *N*-de-ethoxylation of BH 517-TSO), BH 517-T1SO$_2$ and BH 517-T2SO. Only small amounts of unchanged parent compound were detected in the urine.

Toxicological data

Cycloxydim is of low acute toxicity when administered orally, dermally or by inhalation.

The oral LD_{50} of cycloxydim was 3940 mg/kg bw in rats and >5000 mg/kg bw in mice. No specific clinical signs were observed. Macroscopic findings in rats that died after receiving high oral doses by gavage indicated irritation of the gastric mucosa. The dermal LD_{50} in rats was >2000 mg/kg bw, a dose of 2000 mg/kg bw causing neither mortality nor systemic toxicity. No local skin reaction was observed at the application site. When cycloxydim is administered by inhalation, the LC_{50} is >5.28 mg/l of air (4 h exposure). Cycloxydim was not an irritant in a study of ocular and dermal irritation in rabbits or a dermal sensitizer in the Magnusson & Kligman maximization test in guinea-pigs.

Short-term and long-term studies of oral toxicity in mice, rats and dogs were conducted using cycloxydim sodium salt or cycloxydim free acid. In all the studies described below, the dose or dietary concentration of the test substance is expressed as cycloxydim free acid rather than as its sodium salt.

The results of these studies are characterized by clinicochemical changes associated with changes in water and food consumption and effects on the liver. Effects on erythrocytes were seen only in dogs at high doses. Where the test substance was administered in the drinking-water, the reduction in water consumption is regarded to be a palatability effect rather than a specific adverse effect.

With the few available parameters measured in two 4-week range-finding studies in mice, an overall NOAEL was set at 1000 ppm, equal to 189 mg/kg bw per day, on the basis of a significant increase in relative liver weights at concentrations of 3000 ppm and 9000 ppm in combination with altered clinicochemical parameters and the occurrence of hydropic vacuolar parenchymal degeneration of hepatocytes in the first study.

In rats, a 90-day study of oral toxicity indicated that the target organs were the kidney and liver on the basis of increases in concentrations of creatinine, urea and cholesterol in females and increases in the activity of alanine aminotransferase in males and females at 900 ppm. The NOAEL was 300 ppm, equal to 22 mg/kg bw per day.

In the 4-week study of oral toxicity in dogs, the NOAEL was 40 mg/kg bw per day in males on the basis of effects on the liver. The results of a 3-month study of oral toxicity in dogs showed changes in haematological parameters and liver effects, with a NOAEL of 1500 ppm, equal to 50 mg/kg bw per day. In a 1-year study of toxicity in dogs, the NOAEL was 400 ppm, equal to 12 mg/kg bw per day, on the basis of effects on erythrocytes and the liver and altered clinicochemical parameters.

The 2-year study of carcinogenicity in mice did not demonstrate any substance-related change at any drinking-water concentration, and the NOAEL was 240 ppm, equal to 32 mg/kg bw per day (i.e. the highest dose tested). The study was not adequate for the evaluation of carcinogenicity, as the doses delivered were not sufficiently high; the highest dose used was much less than the NOAEL of 1000 ppm identified in the dose range–finding studies.

In an 18-month study in rats, there was a statistically significant reduction in body weight, body weight gain and triglyceride concentrations at drinking-water concentrations of 400 ppm and above, with a NOAEL of 100 ppm, equal to 7 mg/kg bw per day. In a 2-year study of carcinogenicity in rats, administration of drinking-water containing cycloxydim at concentrations of 400 ppm and 1600 ppm resulted in a reduction in body weight. Consumption of drinking-water was reduced in the group at 1600 ppm. In female rats, there was a reduction in concentrations of triglycerides. The NOAEL was 100 ppm, equal to 7 mg/kg bw per day, on the basis of a reduction in body weights and a reduction in concentrations of triglycerides in rats given drinking-water containing cycloxydim at concentrations of 400 ppm and above.

The Meeting concluded that cycloxydim was not carcinogenic in rats but had not been adequately tested in mice.

Cycloxydim was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. Cycloxydim acid and the sodium salt gave negative results throughout, except at cytotoxic concentrations in studies of chromosomal aberration in vitro.

The Meeting concluded that cycloxydim is unlikely to be genotoxic.

Although the carcinogenicity study in mice was not adequate, it was still possible to reach a conclusion on carcinogenicity to humans, in view of the lack of genotoxicity and the absence of carcinogenicity in rats. The Meeting concluded that cycloxydim is unlikely to pose a carcinogenic risk to humans.

In a multigeneration study in rats, the NOAEL for offspring toxicity was 400 ppm, equal to 38 mg/kg bw per day, on the basis of reduced survival, reduced growth and developmental retardation in pups at 1600 ppm, equal to 129 mg/kg bw per day, the highest dose tested. Reproductive toxicity was not affected by treatment at drinking-water concentrations of up to 1600 ppm. The NOAEL for parental toxicity was 100 ppm, equal to 9.7 mg/kg bw per day, on the basis of reductions in food consumption, body weight and body weight gain in dams at 400 ppm.

Studies of developmental toxicity have been carried out in rats and rabbits. In the study of developmental toxicity in rats, the NOAEL for maternal toxicity and embryo/fetotoxicity was 200 mg/kg bw per day. Increased numbers of fetuses/litters with retardations and a statistically significant increase in the frequency of anomalies of the vertebral column and the sternebrae with involvement of the cartilage and incomplete ossification were observed. Maternal toxicity and fetal effects were also observed in two subsequent supplementary studies. In the study of developmental toxicity in rabbits, the NOAEL for maternal toxicity was 100 mg/kg bw per day. The maternal toxicity observed at doses of 200 and 400 mg/kg bw per day occurred late in the study, indicating that repeated dosing over several days was required to elicit the effect. At 400 mg/kg bw per day, the percentage of viable implantations per dam was decreased and the incidence of several skeletal anomalies (e.g. asymmetrical sternebrae and fused sternebrae) was increased above the range for the historical controls. The NOAEL for embryo/fetotoxicity was 200 mg/kg bw per day. The Meeting concluded that cycloxydim causes maternal toxicity that occurred at a late stage during the study. The dose that caused maternal toxicity also caused embryo/fetotoxicity. The Meeting concluded that cycloxydim was not teratogenic.

Some toxicological studies and studies of genotoxicity have been undertaken for four compounds that either are present as impurities in technical cycloxydim or are metabolites in plants and not in animals.

BH 517-5-OH-TSO is of low acute oral toxicity in rats; no mortality or clinical symptoms were observed at the limit dose of 2000 mg/kg bw. Repeated exposure to diets containing BH 517-5-OH-TSO for 90 days did not cause any adverse effects at a dose of 50 mg/kg bw per day. In a 28-day study in rats, the NOAEL for BH 517-$TGSO_2$ was greater than 440.5 mg/kg bw per day, the highest dose tested.

BH 517-TSO, BH 517-5-OH-TSO, BH 517-TGSO and BH 517-$TGSO_2$ were tested for genotoxicity in vitro. All gave negative results.

No reports of adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to cycloxydim were available except for three cases of eye irritation that occurred during production/filling of an old formulation, “Focus Ultra”; after replacement of this formulation by a new formulation, no more such cases have occurred.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.07 mg/kg bw based on the NOAEL of 7 mg/kg bw per day identified on the basis of a reduction in body weights and a reduction in serum triglycerides at concentrations of 400 ppm and above in the long-term dietary study in rats and using a safety factor of 100.

An acute reference dose (ARfD) of 2 mg/kg bw was established for women of childbearing age, based on a NOAEL of 200 mg/kg bw per day identified on the basis of certain skeletal anomalies at 400 mg/kg bw per day in the studies of developmental toxicity in rats and rabbits, and with a safety factor of 100. The Meeting could not exclude the possibility that these skeletal anomalies were the result of a single exposure.

The Meeting concluded that the establishment of an ARfD for the general population was not necessary on the basis of the low acute toxicity of cycloxydim, the lack of evidence for any acute neurotoxicity and absence of any other toxicologically relevant effect that might be attributable to a single dose.

Levels relevant to risk assessment

Species	Study	Effects	NOAEL	LOAEL
Mouse	Two-year study of carcinogenicity[a]	Carcinogenicity	240 ppm, equal to 32 mg/kg bw per day[b]	—
Rat	18-month study of toxicity[a]	Toxicity	100 ppm, equal to 7 mg/kg bw per day	400 ppm, equal to 28 mg/kg bw per day
	Two-year study of toxicity[a]	Carcinogenicity	1600 ppm, equal to 99 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Offspring toxicity	400 ppm, equal to 38 mg/kg bw per day	1600 ppm, equal to 129 mg/kg bw per day
		Reproductive toxicity	1600 ppm, equal to 129 mg/kg bw per day[b]	—
		Parental toxicity	100 ppm, equal to 9.7 mg/kg bw per day	400 ppm, equal to 38 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	200 mg/kg bw per day	400 mg/kg bw per day
		Embryo/fetotoxicity	200 mg/kg bw per day	400 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	100 mg/kg bw per day	200 mg/kg bw per day
		Embryo/fetotoxicity	200 mg/kg bw per day	400 mg/kg bw per day
Dog	One-year study of toxicity[d]	Toxicity	400 ppm, equal to 12 mg/kg bw per day	1600 ppm, equal to 49 mg/kg bw per day

[a] Administration in drinking-water.
[b] Highest dose tested.
[c] Gavage administration.
[d] Dietary administration.

Estimate of acceptable daily intake for humans

0–0.07 mg/kg bw

Estimate of acute reference dose

2 mg/kg bw for women of childbearing age

Unnecessary for the general population

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and almost completely absorbed (>90%) within 24 h
Distribution	Widely distributed; highest concentrations in liver and kidney
Potential for accumulation	No evidence for accumulation
Rate and extent of excretion	About 78–85% of the administered dose is eliminated via the urine within 5 days. Faeces contained approximately 12–25%; enterohepatic recirculation occurred.
Metabolism in animals	Extensive. The major metabolite was the sulfoxide (TSO)
Toxicologically significant compounds (animals, plants and the environment)	Cycloxydim
Acute toxicity	
Rat, LD_{50}, oral	3940 mg/kg bw
Rat, LD_{50}, dermal	>2000 mg/kg bw
Rat, LC_{50}, inhalation	>5.28 mg/l air
Rabbit, dermal irritation	Not an irritant
Rabbit, ocular irritation	Not an irritant
Guinea-pig, dermal sensitization	Not a sensitizer (Magnusson & Kligman test)
Short-term studies of toxicity	
Target/critical effect	Body weight and liver
Lowest relevant oral NOAEL	1000 ppm (189 mg/kg bw per day) (4-week study in mice) 300 ppm (22 mg/kg bw per day) (3-month study in rats) 400 ppm (12 mg/kg bw per day) (1-year study in dogs)
Lowest relevant dermal NOAEL	300 mg/kg bw per day (28-day study in rats)
Genotoxicity	
	Not genotoxic
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body weight
Lowest relevant NOAEL	100 ppm (7 mg/kg bw per day) (rats)
Carcinogenicity	No carcinogenic potential
Reproductive toxicity	
Reproduction target/critical effect	Reduced survival growth and development in pups at parentally toxic doses
Lowest relevant reproductive NOAEL	400 ppm (38 mg/kg bw per day)
Developmental target/critical effect	Increase in the number of skeletal anomalies at maternally toxic doses
Lowest relevant developmental NOAEL	200 mg/kg bw per day (rats and rabbits)

Neurotoxicity/delayed neurotoxicity	
	No data; no concerns raised by other studies
Medical data	
	No significant health effects were reported among manufacturing personnel.

Summary

	Value	Study	Safety factor
ADI	0–0.07 mg/kg bw	Rat, 2-year study	100
ARfD[a]	2 mg/kg bw	Rat and rabbit; study of developmental toxicity	100

[a] For women of childbearing age, unnecessary for the general population.

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FLUOPICOLIDE

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Explanation

Fluopicolide is the International Organization for Standardization (ISO)–approved common name for 2,6-dichloro-*N*-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl] benzamide (International Union of Pure and Applied Chemistry [IUPAC] nomenclature), which has the Chemical Abstracts Service (CAS) No. 239110-15-7. Fluopicolide is a systemic fungicide of the novel chemical class of acylpicolide fungicides and targets oomycetes that cause diseases in a wide range of crops, including potatoes, vegetables and grape vines. Fluopicolide has a new mode of action, which is probably based upon delocalization of spectrin-like proteins. A number of metabolites have been detected in rotational crop studies and are identified in the present document as M-01 (also known as BAM), M-02, M-04 and M-05. Some studies of metabolism and toxicity have been conducted to investigate the properties of these metabolites.

Fluopicolide is being reviewed for the first time by the present Meeting at the request of the Codex Committee on Pesticide Residues. All critical studies complied with good laboratory practice (GLP). Non-GLP studies are identified as such.

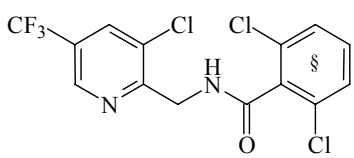
Evaluation for acceptable daily intake

1. Biochemical aspects

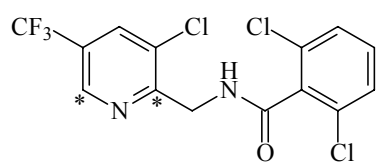
Toxicokinetic studies on the absorption, distribution, metabolism and excretion of fluopicolide (identified in the original studies as AE C638206) were performed in male and female Sprague-Dawley CD rats using two different radiolabelled fluopicolide molecules: either uniformly labelled in the phenyl ring or labelled in the 2 and 6 positions of the pyridine ring (Figure 1). In all studies, the radiochemical purity of fluopicolide was 99% or higher.

Figure 1. Structures of the radiolabelled molecules

[phenyl-U-^{14}C]fluopicolide



[pyridyl-2,6-^{14}C]fluopicolide



§ = uniformly radiolabelled in the phenyl ring

* = radiolabelled in positions 2 and 6 of the pyridine ring

The administrations were single oral doses of either 10 or 100 mg/kg bw (single oral low dose [SOLD] or single oral high dose [SOHD], respectively) or 14 daily doses of 10 mg/kg bw per day (repeated oral low dose [ROLD]). These were preceded by a preliminary study in which single oral doses of 25 or 500 mg/kg bw were used. Some metabolism studies of specific metabolites were also conducted (see section 1.2).

1.1 Absorption, distribution and excretion

The recovery data from the single-dose experiments (Le Lain, 2001; Totis, 2001) demonstrate that the major route of elimination of fluopicolide was via faeces for both dose levels and for both the pyridyl (69–72%) and phenyl (82–88%) radiolabels (Table 1). No significant sex difference was

Table 1. Mean recovery of radioactivity for male and female rats following single and repeated oral administration of [^{14}C]fluopicolide

Sample	% of administered dose							
	Phenyl label				Pyridyl label		Phenyl label	
	Totis (2001)				Le Lain (2001)		Fisher (2004a)	
	SOLD 10 mg/kg bw 168 h post-dose		SOHD 100 mg/kg bw 168 h post-dose		SOLD 10 mg/kg bw 168 h post-dose		ROLD 10 mg/kg bw 144 h post-dose	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine	10.03	13.09	5.39	6.58	18.81	21.37	14.68	21.47
Cage wash	0.96	2.03	1.02	1.76	2.04	5.27	1.66	1.89
Faeces	82.58	82.09	87.46	88.28	72.37	68.78	78.86	72.48
Tissues	1.25	0.99	0.75	1.03	0.66	0.46	0.30	0.46
Total	95.09	98.20	94.60	97.64	93.87	95.88	95.51	96.30

observed. There was a tendency towards a higher urinary excretion level of the pyridyl radiolabel (18.8% in males and 21.4% in females for the 10 mg/kg bw dose) compared with the phenyl radiolabel (10.0% in males and 13.1% in females for the 10 mg/kg bw dose). This suggests that a proportion of the metabolites that were formed differed between the two radiolabels and were presumably linked to the formation of the metabolites 3-chloro-5-(trifluoromethyl)pyridine-2-carboxylic acid (M-02, PCA, AE C657188) from the pyridyl ring moiety and 2,6-dichlorobenzamide (M-01, BAM, AE C653711) from the phenyl ring.

Following 14 daily oral administrations of [phenyl-U-^{14}C]fluopicolide (Fisher, 2004a), the total recovery of radioactivity was approximately 96%, with the faeces again being the major route of elimination, representing 79% of the dose to males and 72% of the dose to females. Recovery of radioactivity from urine represented 15% of the dose to males and 21% of the dose to females. Thus, it appeared that repeated dosing enhanced the elimination via the urine compared with the single oral dosing.

Tissue radioactivity levels at 168 h were consistently low, ranging between 0.46% and 1.25% of the dose for the single-dose studies and with a mean of 0.38% of the dose in the repeated-dose study.

Given the high levels of radioactivity found in the faeces, the biliary elimination of radiolabelled fluopicolide was investigated using both ring-labelled molecules (Totis, 2002; Gutierrez, 2003a). Table 2 presents the percentages of radioactivity eliminated in the first 48 h after dosing for both experiments for the dose rate of 10 mg/kg bw.

These data show that a large proportion of the radioactivity found in the faeces had been absorbed and then eliminated via the bile. By summing the radioactivity levels found in the urine, cage wash, tissues and bile, the extent of oral absorption of [^{14}C]fluopicolide was found to be a mean of 80.06% of the dose for the phenyl radiolabel and 61.53% of the dose for the pyridyl radiolabel. However, blood and plasma pharmacokinetic data show that the systemic exposure was similar between both the radiolabels and the sexes following single oral dosing using either [phenyl-U-^{14}C]-fluopicolide or [pyridyl-2,6-^{14}C]fluopicolide. As these results are in apparent contradiction to the bile excretion study results, a comparison of the recovery data from the bile excretion studies and the equivalent studies performed with non-bile duct–cannulated rats is given in Table 3. The results (derived from Tables 1 and 2) have been normalized to 100% in Table 3.

Table 2. Mean recovery of radioactivity from bile duct–cannulated rats in the first 48 h following single oral administration of [phenyl-U-^{14}C]fluopicolide and [pyridyl-2,6-^{14}C]fluopicolide at the rate of 10 mg/kg bw

Sample	% of administered dose			
	Males		Females	
	Gutierrez (2003a)[a]	Totis (2002)	Gutierrez (2003a)[a]	Totis (2002)
	Pyridyl label	Phenyl label	Pyridyl label	Phenyl label
Urine	5.83	4.55	10.42	6.71
Faeces	40.27	21.48	39.16	19.28
Bile	51.69	70.02	51.74	73.88
Cage wash	0.70	0.77	1.50	0.91
Tissues[a]	2.11	2.03	0.80	1.48
Total absorbed[b]	59.00	77.24	64.05	82.89
Total	100.59	98.85	103.63	102.26

[a] Excluding intestinal and stomach contents.
[b] Sum of radioactivity in urine, cage rinse, bile and tissues.

Table 3. Normalized mean recovery of radioactivity in excreta and tissues following a single oral administration of [pyridyl-2,6-^{14}C]fluopicolide or [phenyl-U-^{14}C]fluopicolide at the rate of 10 mg/kg bw

Sample	% of administered dose (normalized)							
	Males				Females			
	Cannulated		Non-cannulated		Cannulated		Non-cannulated	
	Pyridyl	Phenyl	Pyridyl	Phenyl	Pyridyl	Phenyl	Pyridyl	Phenyl
Urine + cage wash	6.5	5.3	22.2	11.8	11.6	7.5	27.8	15.2
Faeces	40.6	21.6	77.1	86.9	38.2	18.9	71.7	83.8
Bile	52.1	71.1	—	—	49.7	72.3	—	—
Tissues	0.8	1.9	0.7	1.3	0.5	1.4	0.5	1.0
Total	100	100	100	100	100	100	100	100

From Fisher (2007)

A decrease in urinary elimination following bile duct cannulation was observed. By removing the entry of the bile into the intestine, the possibility for reabsorption was eliminated. This implies that there is an active enterohepatic circulation in non-bile duct–cannulated rats. The pyridyl radiolabel appeared to have been more susceptible to this effect than the phenyl radiolabel, suggesting that the fluopicolide molecule has been cleaved. Thus, the enterohepatic recirculation of pyridyl-radiolabelled metabolites is greater than that of the phenyl-radiolabelled metabolites.

It is therefore reasonable to calculate the bioavailability of fluopicolide based upon the results of both the cannulated and non-cannulated rats by using the urinary data from the non-cannulated rats to take into account the material undergoing enterohepatic recirculation and the biliary elimination data from the cannulated rats (tissue data were taken from the non-cannulated rats). This calculation yields mean bioavailability percentages for the pyridyl radiolabel of 75% and 78% of the dose for the males and females, respectively. The mean bioavailability percentages derived from the phenyl radiolabel were 84% and 88% of the dose for the males and females, respectively (Table 4).

Table 4. Normalized mean bioavailability of radioactivity from rats following single oral administration of [^{14}C]fluopicolide at the rate of 10 mg/kg bw

Sample	% of administered dose (normalized)			
	Males		Females	
	Pyridyl label	Phenyl label	Pyridyl label	Phenyl label
Urine + cage wash[a]	22.2	11.8	27.8	15.2
Bile[b]	52.1	71.1	49.7	72.3
Tissues[a,c]	0.7	1.3	0.5	1.0
Total absorbed	75.0	84.3	78.0	88.4

[a] Data taken from non-cannulated rats.
[b] Data taken from cannulated rats.
[c] Excluding intestinal and stomach contents.

Table 5. Summary of the calculated pharmacokinetic parameters following single oral administration of either [pyridyl-2,6-^{14}C]fluopicolide or [phenyl-U-^{14}C]fluopicolide at rates of 10 and 100 mg/kg bw

		C_{max} (µg equiv./g)	T_{max} (h)	$t_{0.5}$ (h)	$AUC_{(0–168\ h)}$ (µg·h/g)	$AUC_{(0–\infty)}$ (µg·h/g)
Whole blood						
Phenyl low dose	Males	1.50 ± 0.24	7.5 ±1	56.63 ±1.61	48.04 ± 8.35	51.65 ± 8.66
	Females	1.19 ± 0.44	5.5 ± 2.52	120.67 ± 26.18	52.87 ± 8.16	73.54 ± 12.62
Pyridyl low dose	Males	1.49 ± 0.51	7.0 ± 1.16	80.34 ± 14.32	40.59 ± 18	45.37 ± 116
	Females	1.18 ± 0.26	6.0 ± 1.63	140.32 ± 25.38	45.22 ± 4.78	67.72 ± 12.92
Phenyl high dose	Males	7.05 ± 1.06	12 ± 8	94.39 ± 9.20	276.83 ± 54.66	311.91 ± 59.94
	Females	6.22 ± 0.57	20 ± 8	124.71 ± 28.47	325.28 ± 42.24	466.91 ± 117.5
Pyridyl high dose	Males	6.34 ± 1.52	8.0 ± 0	79.19 ± 8.12	217.19 ± 28.91	248.56 ± 38.23
	Females	10 ± 0.78	8.0 ± 0	123.84 ± 4.69	244.84 ± 44.51	338.64 ± 69.55
Plasma						
Phenyl low dose	Males	2.20 ± 0.39	8.0 ± 0	18.85 ± 1.49	54.24 ± 10.85	55.22 ± 11.36
	Females	1.61 ± 0.67	6.5 ± 3	19.72 ± 6.21	38.88 ± 9.84	40.28 ± 8.97
Pyridyl low dose	Males	2.14 ± 0.62	7.0 ± 1.16	14.44 ± 2.62	48.39 ± 20.34	48.93 ± 20.32
	Females	1.59 ± 0.23	6.5 ± 1.92	12.67 ± 2.76	30.61 ± 11	30.96 ± 17
Phenyl high dose	Males	9.63 ± 1.72	12 ± 8	13.72 ± 1.73	288.24 ± 72.91	293.64 ± 73.71
	Females	7.03 ± 0.32	20 ± 8	9.52 ± 1.24	224.08 ± 25.98	224.68 ± 26.04
Pyridyl high dose	Males	9.18 ± 2.60	8.0 ± 0	13.48 ± 3.31	229.20 ± 40.10	234.51 ± 40.13
	Females	6.67 ± 1.38	8.0 ± 0	9.39 ± 1.18	175.25 ± 49.23	180.41 ± 49.17

From Fisher & Vinck (2003)
AUC, area under the curve; C_{max}, maximal concentration; equiv., equivalents; T_{max}, time of maximal concentration; $t_{0.5}$, terminal elimination half-life

Following single oral dosing using either [phenyl-U-^{14}C]fluopicolide or [pyridyl-2,6-^{14}C]fluopicolide, the general pharmacokinetic profiles were similar between both the radiolabels and the sexes (Fisher & Vinck, 2003) (Table 5). Radiolabelled fluopicolide was absorbed moderately rapidly, with mean maximal concentrations in blood or plasma being achieved between 7 and 10 h after dosing; this was followed by a moderately rapid elimination, such that the majority was eliminated by 48 h

after dosing, and then a slower terminal elimination phase, with a mean half-life of approximately 103 h. The only biologically significant difference observed was between the dose levels. The difference between the high and low dose levels was that the increase in the total systemic exposure (as measured by the area under the curve from time zero to infinity [$AUC_{(0-\infty)}$]) did not increase proportionately with the dose (approximately 5- to 6-fold versus a 10- to 11-fold increase in dose). This was apparently due to a proportionately lower level of absorption at 100 mg/kg bw, as the mean maximal concentrations (C_{max} values) were also proportionately lower.

The concentrations of radioactivity in the tissues at 168 h following single oral administrations of [phenyl-U-^{14}C]fluopicolide at 10 and 100 mg/kg bw were consistently highest in the liver and the kidneys, with the concentrations in skin and fur also being higher in the females of the high dose group. The mean (males and females) highest concentration observed was 0.37 µg equivalents (equiv.) per gram (liver) for the 10 mg/kg bw group and 1.45 µg equiv./g (liver) for the 100 mg/kg bw group. Tissue concentrations increased, on average, by 3–5 times between the 10 and 100 mg/kg bw dose groups. This finding suggests that the 100 mg/kg bw dose level may have passed the threshold for maximal absorption, a proposition that is supported by the data obtained in the bile excretion and the blood/plasma kinetic studies (Totis, 2001).

Following single oral administration of [pyridyl-2,6-^{14}C]fluopicolide to male and female rats at the rate of 10 mg/kg bw, the organs containing the highest concentrations 168 h later were most consistently the liver, kidneys, spleen and blood. The highest mean concentrations were observed in the kidneys for the males (0.23 µg equiv./g) and the cardiac blood for the females (0.30 µg equiv./g). The concentrations in the majority of the other tissues were below 0.1 µg equiv./g. No detectable radioactivity was observed in the thyroid samples from either sex (Le Lain, 2001).

Following repeated oral administration of [phenyl-U-^{14}C]fluopicolide to male and female rats at the rate of 10 mg/kg bw, the organs containing the highest concentrations 144 h later were most consistently the liver (1.57 µg equiv./g), kidneys and cardiac blood (Fisher, 2004a).

In a tissue kinetic study of [phenyl-U-^{14}C]fluopicolide, radioactivity was rapidly and widely distributed to the tissues following single oral doses at the nominal rates of 10 and 100 mg/kg bw when sampled at 8, 24, 36 and 72 h after dosing for males and 8, 30, 48 and 120 h after dosing for females (Fisher, 2003a). Data from an earlier study (Totis, 2001) were used to provide tissue concentrations at 168 h after dosing.

At the earliest sampling time (8 h; Fisher, 2003a), the highest mean concentrations of radioactivity in tissues in male rats following the single 10 mg/kg bw dose of [phenyl-U-^{14}C]fluopicolide were found in the intestine and contents (53.71 µg equiv./g), followed by the liver (5.93 µg equiv./g), adrenals (5.17 µg equiv./g), kidneys (4.21 µg equiv./g), fat (3.73 µg equiv./g), plasma (3.47 µg equiv./g) and blood (2.26 µg equiv./g). The thyroids, lungs, heart, Harderian gland, pancreas, skin and fur, spleen, testes and carcass all had mean concentrations between 1 and 2 µg equiv./g. In muscle, stomach and contents, bone and marrow, eyes and brain, mean concentrations were between 0.5 and 1 µg equiv./g. At the same sampling time, the highest mean concentrations of radioactivity in tissues in female rats were found in the intestine and contents (69.31 µg equiv./g), followed by fat (10.86 µg equiv./g), stomach and contents (6.70 µg equiv./g), adrenals (5.37 µg equiv./g), liver (4.88 µg equiv./g), kidneys (4.72 µg equiv./g) and thyroids (3.25 µg equiv./g). The uterus, ovaries and plasma were found to possess levels between 2.33 and 2.77 µg equiv./g. The lungs, heart, Harderian gland, pancreas, skin and fur, spleen, blood and carcass all had mean concentrations between 0.97 and 1.87 µg equiv./g. In muscle, bone and marrow, eyes and brain, mean concentrations were between 0.32 and 0.54 µg equiv./g.

Between the first and second sampling times (at 24 h for males and at 30 h for females), concentration decline was rapid in most tissues, but the concentrations declined less rapidly in liver and kidney than in other tissues. Expressed as percentages of the earliest sampling time value, the

concentrations in males and females, respectively, were as follows: at 24 h/30 h, liver, 49% and 39%; kidney, 58% and 29%; and at 36 h/48 h, liver, 40% and 22%; kidney, 40% and 17%.

Levels in the tissues from the male and female rats at 168 h after a single dose of 10 mg/kg bw had decreased by means of 95.2% ± 3.5% and 95.1% ± 3.9%, respectively, from the concentrations observed at 8 h after dosing.

At the earliest sampling time (8 h; Fisher, 2003a), the highest mean concentrations of radioactivity in tissues in male rats following the single 100 mg/kg bw dose of [phenyl-U-^{14}C]fluopicolide were found in the intestine and contents (594.42 µg equiv./g), followed by fat (22.03 µg equiv./g), liver (17.69 µg equiv./g), adrenals (14.32 µg equiv./g), stomach and contents (14.02 µg equiv./g) and kidneys (13.30 µg equiv./g). In all other tissues, the mean concentrations were below 10 µg equiv./g. The lowest mean concentrations at 8 h were observed in both the bone and marrow and the eyes, at 2.11 µg equiv./g. At the same sampling time, the highest mean concentrations of radioactivity in tissues in female rats were found in the intestine and contents (843.16 µg equiv./g), followed by the stomach and contents (94.95 µg equiv./g), fat (59.41 µg equiv./g), liver (18.24 µg equiv./g), adrenals (18.13 µg equiv./g) and kidneys (17.61 µg equiv./g). The ovaries, Harderian gland, pancreas and skin and fur all had between 10.17 and 14.24 µg equiv./g. In all remaining tissues, mean concentrations were below 10 µg equiv./g. The lowest mean concentrations at 8 h were observed in bone and marrow and the eyes, at 2.05 µg equiv./g and 1.21 µg equiv./g, respectively.

The concentration decline with time after the 100 mg/kg bw dose was similar to that observed after the 10 mg/kg bw dose. The difference in achieved concentrations in the tissues between the two dose levels was not dose proportional, indicating that proportionately less of the administered radioactivity was absorbed at the higher dose rate.

In a tissue kinetic study of [2,6-pyridyl-^{14}C]fluopicolide, radioactivity was rapidly and widely distributed to the tissues following a single oral dose at the nominal rate of 10 mg/kg bw when sampled at 7, 24, 36, 48 h after dosing for males and 6, 24, 36 and 120 h after dosing for females (Fisher, 2003b). Data from an earlier study (Le Lain, 2001) were used to provide tissue concentrations at 168 h after dosing. The highest radioactivity concentrations were observed in the intestine and contents at all sampling times for the males and up to 36 h after dosing for the females; this probably reflects the presence of biliary excretion of radioactivity. At the earliest sampling time (7 h), in both males and females, the highest concentrations in tissues (expressed as µg [2,6-pyridyl-^{14}C]fluopicolide equivalents per gram tissue) were found in fat (males 5.8; females 12.1), adrenals (males 5.4; females 5.8) and liver (males 4.6, females 4.4). There were also appreciable concentrations in kidney (males 2.8; females 4.2), pancreas (males 2.3; females 2.9) and ovaries (2.9). Thereafter, a significant and rapid decrease in tissue concentrations was observed, with an approximately 96% decrease (mean) between the earliest sampling time 7 h and 6 h after dosing in males and females, respectively, and 168 h after dosing. Concentration decline was rapid in most tissues within the first 24 h, but the concentrations declined less rapidly in liver and kidney than in other tissues. Expressed as percentages of the earliest sampling time value, the concentrations in males and females, respectively, were as follows: at 24 h, liver, 43% and 47%; kidney, 28% and 26%; and at 36 h, liver, 36% and 34%; kidney, 26% and 19%. There did not appear to be a significant sex difference.

1.2 Metabolism

The investigations into the metabolism of fluopicolide all demonstrated that it was capable of being extensively metabolized by the rat (Fisher, 2003a, 2004b,c,d). In spite of the large number of metabolites found, the basic fluopicolide structure remained intact in the majority of cases. In the liver at 8 h after dosing in the tissue kinetic study with [phenyl-U-^{14}C]fluopicolide described above (Fisher, 2003a), up to 13 different radioactive fractions were found. Five of these fractions were identified and quantified as percentages of the administered dose (males and females, respectively) as AE

C653711 (M-01, BAM) (0.09% and 0.08%), AE 0717559 (0.02% and 0.01%), AE C643890 (0.03% and 0.10%), AE 0717560 (0.08% and 0.09%) and fluopicolide (0.04% and 0.20%).

In metabolic studies of fluopicolide in excreta of rats, up to 46 radioactive components were found in the urine and up to 14 radioactive components were found in the faeces of males and females, taken together. Important reactions were hydroxylation of the phenyl ring in two positions, which could then be conjugated to form glucuronides or sulfates; and substitution of the chlorine atom on the phenyl ring by glutathione (catalysed by glutathione *S*-transferase), followed by degradation to the cysteine conjugate. These conjugates could then be cleaved via β-elimination by cysteine conjugate β-lyases, leaving a resulting thiol that could serve as the methyl acceptor—a mechanism known as the thiomethyl shunt. The resulting *S*-methyl group could then be oxidized to provide sulfone and sulfoxide structures. In contrast to these multiple reactions of the phenyl moiety, the pyridyl structure appeared to be metabolically stable. Scission of fluopicolide resulted in the formation of M-01 and M-02. The former was further metabolized in a manner similar to the phenyl ring of intact fluopicolide, whereas the latter remained intact and appeared to undergo only conjugation reactions at the newly formed carboxylic group.

Following a single oral administration of [phenyl-U-^{14}C]fluopicolide, up to 33 different radioactive fractions were observed in the urine from male rats, and up to 42 fractions were found in female rat urine. Very few of these metabolites exceeded 1% of the dose. The highest single fraction was found in female rat urine and represented 1.534% of the dose. This was a thiomethyl derivative that was also conjugated with sulfate after hydroxylation of the phenyl ring. In faeces, up to 11 components were found in males and females, but there was a single large contributor that represented about 80% of the dose; this was unchanged fluopicolide.

The metabolite profile observed in the faecal extracts was therefore similar between the sexes.

Investigations of the metabolism of [pyridyl-2,6-^{14}C]fluopicolide in rats revealed the presence of up to 28 radioactive components in the urine and up to 31 radioactive components in the faecal extracts (Fisher, 2004b). In that study, investigations by liquid chromatography with mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS) permitted a total of 92.88% of the radioactivity eliminated in the urine by the male rats to be assigned a proposed structure, which accounted for 16.19% of the administered dose. For the females, a total of 82.6% of the radioactivity eliminated in the urine has been assigned a proposed structure, which accounted for 17.10% of the administered dose.

A proposed metabolic pathway for fluopicolide is presented in Figures 2–4.

2. Toxicological studies

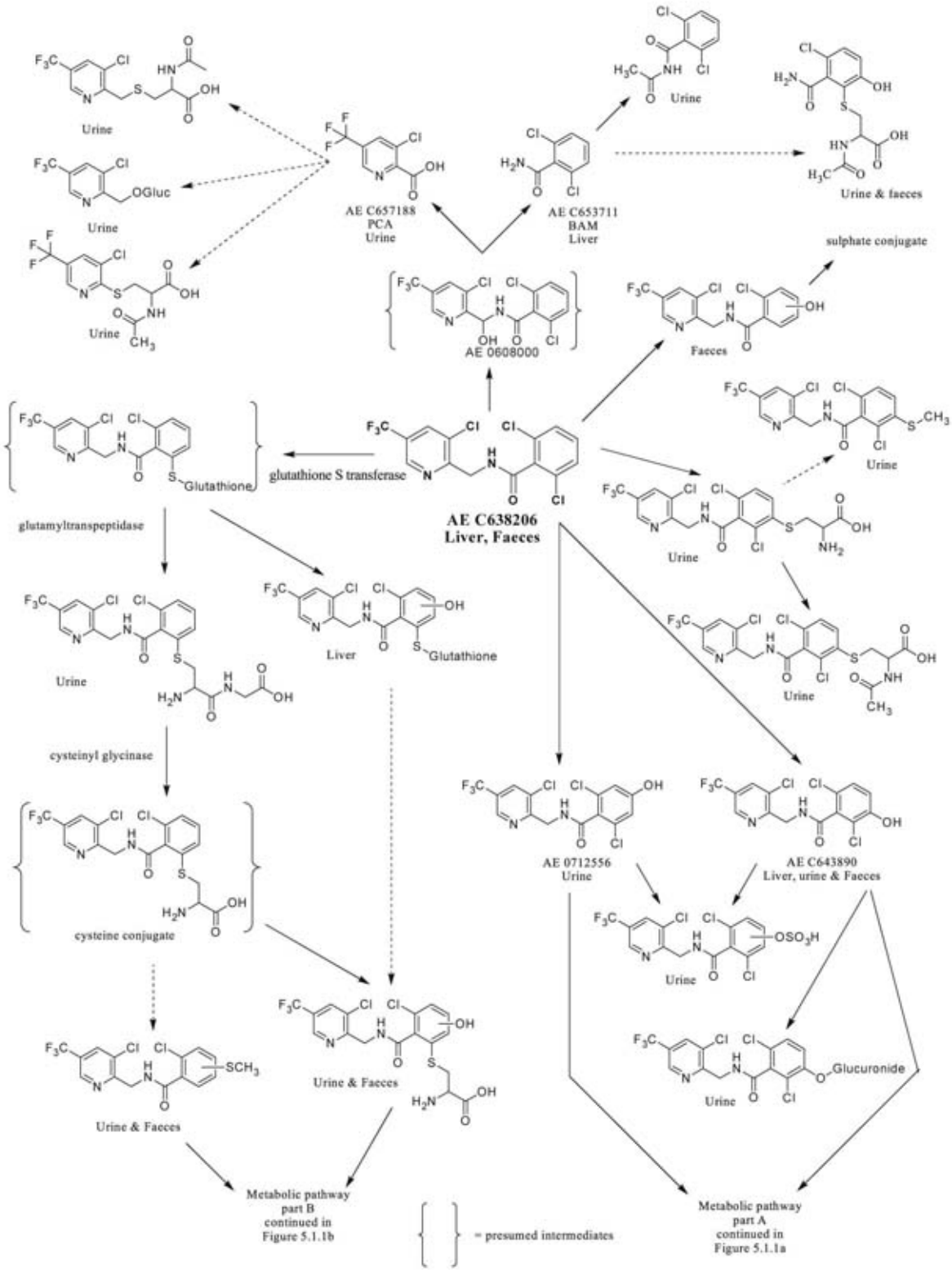
2.1 Acute toxicity

(a) Oral administration

In an acute oral toxicity study conducted according to Organisation for Economic Co-operation and Development (OECD) test guideline 423 (adopted in 1996), five male and five female fasted Hsd Sprague-Dawley (CD) rats were administered fluopicolide (purity 97.7% by weight [w/w]; batch Nos PP/241024/2 and PP/241067/1) in 1% weight per volume (w/v) aqueous methyl cellulose (at a concentration of 25.5% w/v; application volume 20 ml/kg bw) by gavage as a single oral dose of 5000 mg/kg bw. Mortality, clinical signs and body weight changes were recorded. Autopsies were performed on rats at termination on day 15.

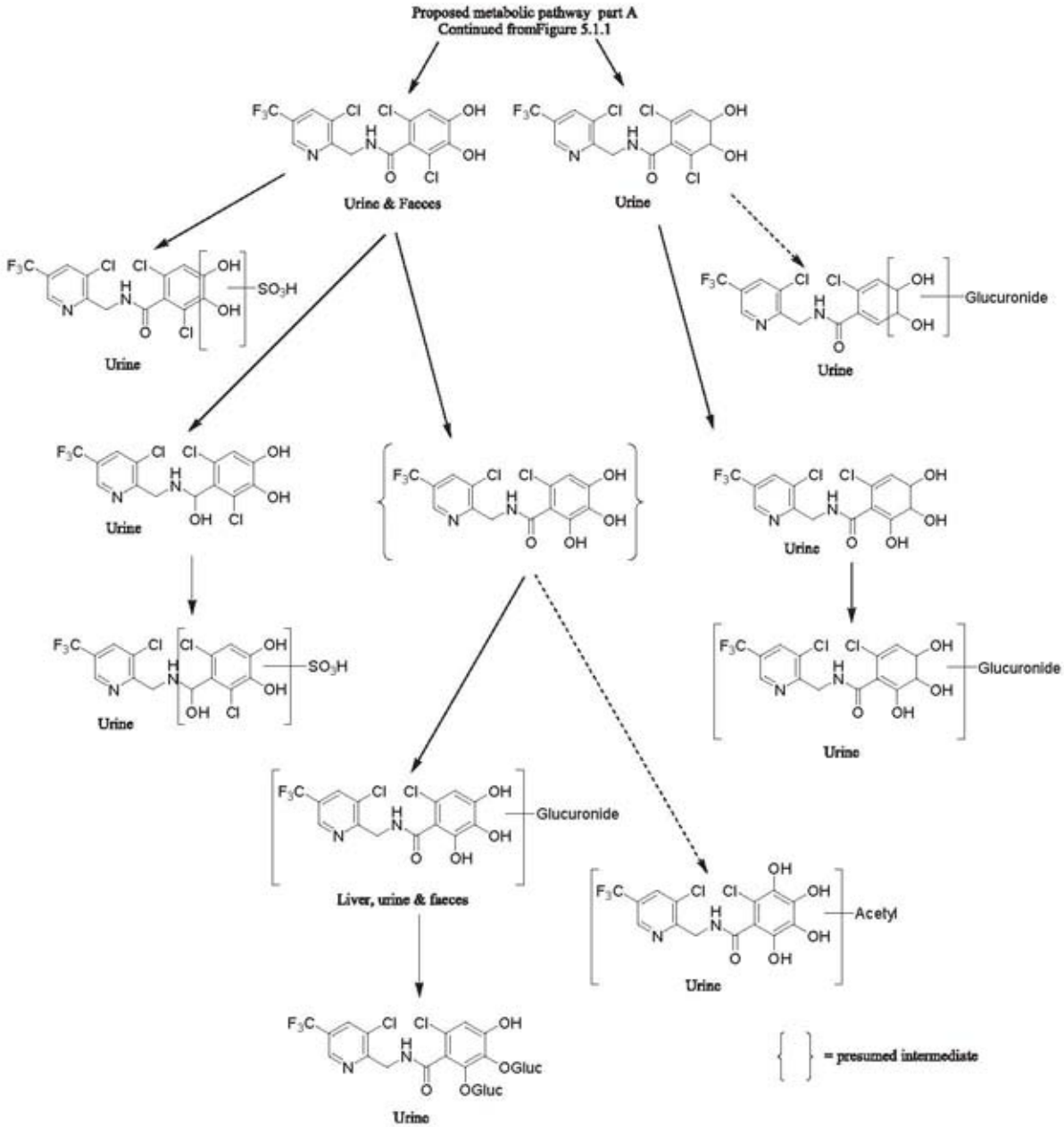
There were no deaths in the study following a fluopicolide dose of 5000 mg/kg bw. Clinical signs of toxicity included piloerection within 1–2.5 h after dosing in all rats. Later on day 1,

Figure 2. A proposed metabolic pathway for fluopicolide (AE C638206) in rats



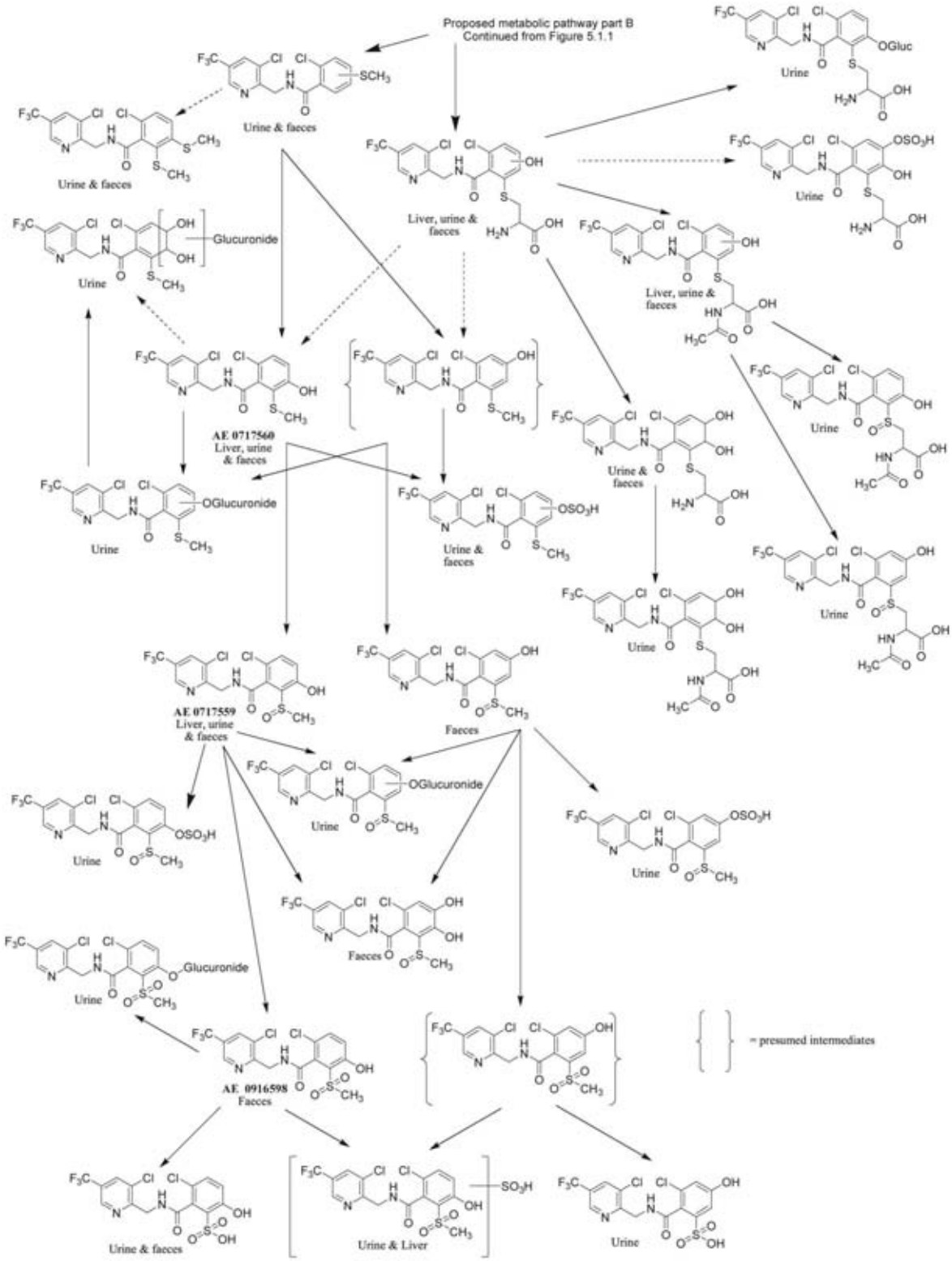
Note: Figures 5.1.1a and 5.1.1b are Figures 3 and 4 in this monograph

Figure 3. A proposed metabolic pathway for fluopicolide in rats (continued)



Note: Figure 5.1.1 is Figure 2 in this monograph

Figure 4. A proposed metabolic pathway for fluopicolide in rats (continued)



Note: Figure 5.1.1 is Figure 2 in this monograph

piloerection was accompanied only by hunched posture (five females and three males) and abnormal gait (three females only). Recovery was complete by day 3. All rats were considered to have achieved satisfactory body weight gains during the study. Autopsies performed on day 15 did not reveal any gross abnormalities.

The acute oral median lethal dose (LD_{50}) of fluopicolide in rats was >5000 mg/kg bw (McRae, 2000a).

(b) Dermal application

In an acute dermal toxicity study conducted according to OECD test guideline 402 (adopted in 1987), five male and five female Sprague-Dawley (CD) rats were administered fluopicolide (purity 97.7%; batch Nos PP/241024/2 and PP/241067/1) to the closely clipped dorsum at a dose of 5000 mg/kg bw. The applied fluopicolide was covered by an occlusive dressing for 24 h. Local or systemic signs of reaction to treatment were recorded during a subsequent 14-day period of observation. All rats were killed on day 15 and subjected to gross postmortem examination.

There were no deaths or clinical signs of reaction to treatment in any rat throughout the study. There was no evidence of a dermal response to treatment throughout the study. A slight reduction in body weight was evident in two of five females on day 8. All other rats were considered to have achieved satisfactory body weight gains throughout the study. Autopsies performed on day 15 did not reveal any gross abnormalities.

The acute dermal LD_{50} of fluopicolide in rats was >5000 mg/kg bw (McRae, 2000b).

(c) Inhalation

In an acute inhalation study conducted according to OECD test guideline 403 (adopted in 1981), five male and five female Sprague-Dawley Crl:CD BR strain rats were exposed (nose only) for 4 h to a dust aerosol of fluopicolide (purity 98.3%; batch Nos PP/241024/2 and PP/241067/1) at an analytical concentration of 5.16 mg/l (nominal concentration 9.09 mg/l). The rats were observed for 14 days after exposure, then killed and autopsied. Parameters monitored included mortality, clinical signs and weekly body weights.

The characteristics of the achieved atmosphere were as follows: mean achieved atmospheric concentration, 5.16 ± 0.38 mg/l; mass median aerodynamic diameter (MMAD), 3.37 μm; and geometric standard deviation, 2.09. The respirable fraction (percentage <4 μm) was 59%.

There was no mortality during the exposure and observation period. Common observations noted both during and after exposure included wet fur, hunched posture, piloerection and increased respiratory rate. Isolated occurrences of noisy respiration and red/brown staining around the snout or eyes were also seen. Rats recovered quickly, appearing normal on the first day after exposure. Normal body weight gain was noted during the study. No gross abnormalities were noted for 9 of 10 rats. One male showed dark foci on its lungs.

The 4 h acute inhalation median lethal concentration (LC_{50}) of fluopicolide in rats was >5.16 mg/l (the mean achieved concentration) (Wesson, 2000).

(d) Dermal irritation

In a dermal irritancy study conducted according to OECD test guideline 404 (adopted in 1992), three albino New Zealand White rabbits were exposed to 0.5 g of fluopicolide (purity 97.7%; batch Nos PP/241024/2 and PP/241067/1) under an occlusive dressing on the closely clipped dorsal skin for 4 h. Dermal reactions were assessed at 1, 24, 48 and 72 h after removal of the dressing.

No dermal irritation was observed during the study. The mean irritation score over 24–72 h was 0 for erythema and 0 for oedema. Fluopicolide was not irritating to rabbit skin (McRae, 2000c).

(e) Eye irritation

In an eye irritation study conducted according to OECD test guideline 405 (adopted in 1992), four New Zealand White rabbits were exposed to 0.1 ml of fluopicolide (purity 97.7%; batch Nos PP/241024/2 and PP/241067/1) instilled into the conjunctival sac of one eye only on day 1. Ocular reactions were assessed at 1, 24, 48 and 72 h and 7 days after treatment. In a preliminary screen conducted with one of the four rabbits, the eye was rinsed 30 s after instillation. The treated eye of each of the other three rabbits was not rinsed.

The single instillation of fluopicolide into the eye of the screen rabbit, followed by rinsing, provoked slight conjunctival irritation observed 1 h after instillation, but not at the next observation time, 24 h. The single instillations of fluopicolide into one eye of each of the three main test rabbits, not followed by rinsing, elicited slight conjunctival irritation in all rabbits from 1 h after instillation. The ocular reactions resolved in all instances within 2 days after instillation.

Fluopicolide was transiently slightly irritating to the rabbit eye (McRae, 2000d).

(f) Dermal sensitization

In a skin sensitization study conducted according to OECD test guideline 406 (adopted in 1992), delayed-contact hypersensitivity in guinea-pigs exposed to fluopicolide (purity 97.7%; batch Nos PP/241024/2 and PP/241067/1) was assessed by the Magnusson and Kligman maximization method. Based on the findings of a preliminary study, the closely clipped dorsa of 10 male and 10 female Dunkin Hartley guinea-pigs were given intradermal injections of Freund's complete adjuvant, 10% w/v fluopicolide in sterile water and 10% w/v fluopicolide in a 50:50 mixture of Freund's complete adjuvant in sterile water on day 1. Six days later, the same area of skin was treated by topical application of 100% w/v fluopicolide in sterile water, and the test site was covered by an occlusive dressing for 48 h. The same induction procedures were carried out on 10 control group guinea-pigs, except that the test material was replaced by vehicle. Two weeks after the topical induction, all guinea-pigs were challenged by occluded application of 100% fluopicolide in sterile water to the anterior site on the flank and 50% fluopicolide in sterile water to the posterior site on the flank. The occlusive dressings were removed on the following day, and the condition of the test sites was assessed approximately 24 and 48 h later.

There were no deaths or signs of ill-health or toxicity. Body weight changes were similar between control and treated guinea-pigs. Necrosis was observed at sites receiving Freund's complete adjuvant in all test and control guinea-pigs following intradermal injections. Slight irritation was seen in 6 of 20 guinea-pigs receiving 10% w/v fluopicolide in sterile water, and no irritation was observed in controls. Following topical application, slight to well-defined erythema was observed in all test guinea-pigs receiving 100% w/v fluopicolide.

The challenge application produced no dermal reactions indicative of skin sensitization in any of the test or control guinea-pigs. Slight erythema was observed in two test guinea-pigs at the 24 and 48 h readings compared with slight to well-defined erythema for two control guinea-pigs at the 48 h reading only. The reactions observed were noted to be of similar incidence and severity. As no reactions were observed for any of the remaining test or control guinea-pigs, the overall response was considered negative.

Fluopicolide was not a skin sensitizer in this guinea-pig Magnusson and Kligman test (McRae, 2000e).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Groups of five male and five female CrlCD-1 (ICR) BR mice were administered fluopicolide (purity 99.0%; batch No. CDB234187-1) in the diet at nominal concentrations of 0, 6, 64, 640 or 6400 ppm (equal to 0, 0.95, 10.4, 100 and 980 mg/kg bw per day in males and 0, 1.19, 12.9, 129 and 1242 mg/kg bw per day in females) for a minimum of 28 consecutive days. Mice were observed daily for clinical signs, whereas body weight and food consumption were measured weekly. Haematology and blood chemistry examinations were carried out at the end of treatment. At necropsy, the weights of selected organs were recorded; subsequently, microscopic examinations were performed on specified organs and tissues.

The mean analytical results for the test diet samples were within the range 90.5–101.0% of nominal (the laboratory's acceptable range +10% to −15% of nominal). Homogeneity was satisfactory at all levels; that is, mean values obtained for top, middle and bottom samples were within the acceptable range of 90–110% of nominal, and these mean percentage of nominal values differed by less than 10%. Stability was satisfactory over the time of use of the diet (8 days); in other words, nominal levels declined by a maximum of 12% over 8 days of storage at room temperature.

There were no mortalities, no treatment-related clinical signs and no body weight or food consumption changes throughout the study at any dose levels. Haematological parameters did not show any treatment-related changes compared with controls. Blood chemistry analysis showed increased alanine aminotransferase activity in males at 640 ppm (181%, $P < 0.01$) and 6400 ppm (247%, $P < 0.001$). Alkaline phosphatase activity was also increased in male mice at 6400 ppm (231%), which is considered to be suggestive of possible impairment of liver function in males.

Increases in absolute liver weight of 33% and 50% were observed in males and females, respectively, at 6400 ppm, whereas liver weight relative to body weight was also increased by 42% and 58% in males and females, respectively, compared with controls. At 640 ppm, there were increases of 19% in absolute and relative liver weights in both sexes. Microscopic examination of the liver revealed increased centrilobular hypertrophy at 6400 ppm in all males and females and at 640 ppm in all males and four of five females.

The no-observed-adverse-effect level (NOAEL) in the 28-day dietary study in mice was 64 ppm, equal to 10.4 mg/kg bw per day, based on increased liver weight and centrilobular hepatocyte hypertrophy at a dose level of 640 ppm, equal to 100 mg/kg bw per day (Mallyon, 2000a).

In a 90-day dietary toxicity study in mice, groups of 10 male and 10 female Crl:CD-1 (ICR) BR mice were administered fluopicolide (purities 96.9% and 97.3% for batch Nos TM99002129 and TM99002622, respectively) in the diet at nominal concentrations of 0, 32, 320, 3200 or 6400 ppm, equivalent to 0, 5.5, 53, 545 or 1092 mg/kg bw per day, for at least 90 days. Clinical signs were recorded daily, and body weights and food consumption were measured weekly. Blood samples were collected during week 13 from the retro-orbital venous plexus of each mouse for selected blood chemistry measurements and haematology. All mice were killed at termination and subjected to post-mortem examination, selected organs were weighed, and a range of tissues were taken, fixed and examined microscopically.

The stability of fluopicolide in the diet was demonstrated in the prestudy mix of 25 and 10 000 ppm samples, which diets were either kept at ambient temperature for up to 56 days or frozen for 7 weeks at below −15 °C and then kept at ambient temperature for 1 week. The homogeneity of fluopicolide in the diet was verified for the lowest and highest concentrations from the first study diet mix. Dietary levels of the test substance were verified for each concentration. Excessive food

spillages were noted for the 3200 ppm dose groups during weeks 5 and 7 for males and weeks 5, 6, 7 and 9 for females; consequently, achieved intakes could not be calculated for these times.

There were no mortalities or clinical signs of toxicity. There was no clear treatment-related effect on food consumption. Overall body weight gain was reduced in both males (20%) and females (32%) at 6400 ppm and females only (22%) at 3200 ppm. There were no significant body weight gain reductions at any other dose level. Blood chemistry assessment revealed that alanine aminotransferase activity was slightly increased in both sexes at 6400 and 3200 ppm, whereas aspartate aminotransferase activity was slightly increased in males only at 6400 and 3200 ppm. Slight increases in total cholesterol and creatinine concentrations were observed in females at 6400 ppm, at which dose there was also a slight increase in alkaline phosphatase activity in males.

Absolute liver weights were increased by 42% in males and 60% in females at 6400 ppm and by 33% in males and 44% in females at 3200 ppm. Liver weight relative to body weight increased by 50% in males and 78% in females at 6400 ppm and by 36% in males and 44% in females at 3200 ppm. There were no significant changes in liver weight at the 320 or 32 ppm doses.

At the terminal necropsy, abnormal areas of the liver were noted in 2 of 10 males and 3 of 10 females at 6400 ppm and in 3 of 10 males and 3 of 10 females at 3200 ppm. Microscopic examination revealed centrilobular hepatocellular hypertrophy in all male and female mice at 6400 ppm, scored as slight to severe in males and slight to moderate in females; at 3200 ppm, centrilobular hepatocellular hypertrophy of minimal to moderate severity occurred in all male mice and in 9 of 10 females. Focal hepatocytic necrosis of minimal to moderate severity occurred in 3 of 10 males and 2 of 10 females at 6400 ppm and of slight severity in 2 of 10 females (not in males) at 3200 ppm.

The NOAEL in the 90-day dietary study in CD-1 mice was 32 ppm, equivalent to 5.5 mg/kg bw per day, based on liver histopathology at 320 ppm, equivalent to 53 mg/kg bw per day (Mallyon, 2000b).

At this point, the decision was taken to change the strain of mouse used in these studies to C57BL/6JICO; therefore, the 90-day study in mice was repeated using this different mouse strain.

In a 90-day dietary toxicity study in mice, groups of 10 male and 10 female C57BL/6JICO mice were administered fluopicolide (purity 95.9%; batch No. 0P2050046) in the diet at nominal concentrations of 0, 50, 200, 800 and 3200 ppm, equal to 0, 10.4, 37.8, 161 and 770 mg/kg bw per day in males and 0, 12.6, 52.8, 207 and 965 mg/kg bw per day in females, for at least 90 days. These dose levels were based on the previous 90-day toxicity study performed in CD-1 mice (Mallyon, 2000b). Clinical signs were recorded daily, and body weights and food consumption were measured weekly. On the day of necropsy, blood samples were collected from the retro-orbital venous plexus of each mouse for selected blood chemistry measurements, which included total bilirubin, urea, total protein, albumin and total cholesterol concentrations, and aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities. All mice, either found dead or killed at termination, were subjected to postmortem examination, selected organs were weighed, and a range of tissues was taken, fixed and examined microscopically.

The stability of fluopicolide in the diet was demonstrated in the prestudy mix of 25 and 10 000 ppm samples, which diets were either kept at ambient temperature for up to 56 days or frozen for 7 weeks at below −15 °C and then kept at ambient temperature for 1 week. The homogeneity of fluopicolide in the diet was verified for the lowest and highest concentrations from the first study diet mix. Dietary levels of the test substance were verified for each concentration. Excessive food spillages were noted for the 3200 ppm dose groups during weeks 5 and 7 for males and weeks 5, 6, 7 and 9 for females; consequently, achieved intakes could not be calculated for these times. Four mice were found dead or killed while in a moribund condition between days 6 and 73: one male from each of the 0, 200 and 3200 ppm groups and one female from the 800 ppm group. In addition, four male

Table 6. Summary of certain blood chemistry data from male and female mice at termination in the 90-day dietary study of fluopicolide

Parameter	Males				Females			
	Dietary concentration (ppm)							
	0	200	800	3200	0	200	800	3200
Total cholesterol (mmol/l)	1.82	1.36	0.95	0.91	1.51	1.19	1.16	1.27
Albumin (g/l)	38	37	33	33	38	37	34	33

From Wason (2001)

mice died from accidental traumas between days 21 and 85: one from the 0 ppm group, two from the 800 ppm group and one from the 3200 ppm group. On day 94, one control female and one female at 50 ppm died during anaesthesia. All the unscheduled deaths were considered to be incidental and not related to treatment.

There were no treatment-related clinical signs. There was no clear treatment-related effect on feed consumption. Overall body weight gain was slightly reduced in both males (7%) and females (14%) at 3200 ppm. The effect on body weight gain was most marked during week 1 for males (−88%, $P = 0.01$) and weeks 1 and 2 for females (−85% to −87%, $P = 0.05$–0.01). Absolute body weights in the 3200 ppm group were reduced by 10% in males and 7% in females after 1 week of treatment. Terminal body weights were slightly reduced for males at 3200 ppm (−5%, $P = 0.05$). There were no significant body weight gain reductions at any other dose level. Blood chemistry assessment (Table 6) revealed decreases in total cholesterol concentration in both sexes at 3200, 800 and 200 ppm, although it is not clear that the changes in females were dose related. Small decreases in albumin concentration of up to 13% were observed in both sexes at 3200 and 800 ppm. Mean alkaline phosphatase activity was slightly increased (+29%, $P < 0.01$) in males at 3200 ppm.

Absolute liver weights were increased by 20% in males and 25% in females at 3200 ppm, and relative (to body weight) liver weight increased by 24–30% in males and 34% in females. Both absolute and relative liver weights were slightly increased by 10–14% in males and 13–16% in females at 800 ppm, but there were no significant changes in liver weights at lower doses.

Gross examination of mice that died revealed no treatment-related changes. At the terminal necropsy, dark liver was noted in 4 of 8 males and 9 of 10 females at 3200 ppm. Microscopic examination revealed diffuse centrilobular hepatocellular hypertrophy in one decedent male at 3200 ppm and one decedent female at 800 ppm, as well as in all surviving 3200 ppm group mice and the majority of mice at 800 ppm (Table 7). At terminal examination, the severity of these hepatocytic changes in the 3200 ppm group ranged from slight in one male mouse to mild in the remaining 7 male mice and all 10 female mice. At 800 ppm, severity was graded slight in 4 of 8 male mice and 8 of 8 female mice.

The NOAEL in the 90-day dietary study in mice was 50 ppm, equal to 10.4 mg/kg bw per day, based on a significant treatment-related reduction in cholesterol of male mice indicative of impaired liver function at 200 ppm, equal to 37.8 mg/kg bw per day (Wason, 2001).

Taking account of both of these 90-day toxicity studies in mice of different strains, the overall NOAEL was 50 ppm, equal to 10.4 mg/kg bw per day, based on serum chemistry suggesting impaired liver function at 200 ppm, equal to 37.8 mg/kg bw per day, in the second strain and histopathology of the liver at 320 ppm, equivalent to 53 mg/kg bw per day, in the first strain.

Table 7. Summary of the microscopic changes in the liver of male and female mice at termination in the 90-day dietary study of fluopicolide

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	50	200	800	3200	0	50	200	800	3200
No. of mice examined	8	10	9	8	8	9	9	10	9	10
Hypertrophy, hepatocellular, centrilobular, diffuse										
- Slight	0	0	0	4	1	0	0	0	8	0
- Mild	0	0	0	0	7	0	0	0	0	10
- Total	0	0	0	4	8	0	0	0	8	10

From Wason (2001)

Rats

In an oral study of toxicity, groups of five male and five female Sprague-Dawley Crl:(IGS) CDBR rats were administered fluopicolide (purity 99.9%; batch No. CDB234167-2) in the diet at dose levels of 0, 20, 200, 2000 or 20 000 ppm, equal to 0, 1.74, 17.4, 174 and 1720 mg/kg bw per day in males and 0, 1.81, 17.9, 184 and 1820 mg/kg bw per day in females, for a minimum of 28 consecutive days. Rats were observed daily for clinical signs of toxicity and mortality. Once before exposure and again on study day 2 and weekly thereafter, a functional observational battery (FOB) of tests, including grip strength assessment, was conducted. In week 4, in addition to these tests, motor activity was assessed. The FOB consisted of a combination of examinations that assess the reaction of rats to handling on removal from the cage and observations in an open field standard arena. Motor activity was measured by a video tracking, motion analysis and behaviour recognition system to derive the total distance moved (in centimetres) by each rat during a 60 min period. Body weight and food consumption were measured weekly, and water consumption was measured during the third week of treatment. Haematology and blood chemistry investigations were conducted on blood samples collected on day 28. Eye examinations were carried out before treatment started and at the end of the study. At necropsy, the weights of selected organs were recorded; subsequently, a range of tissues was examined histopathologically.

Test diets were prepared weekly throughout the study. Test formulations were analysed for stability and concentration of fluopicolide. The mean analytical results for the test diet samples were within the range 92.9–100.9% of nominal (acceptable range was considered to be +10% to −15% of nominal). Stability was satisfactory over the time of use of the diet (8 days); that is, nominal levels declined by a maximum of 7% over 8 days of storage at room temperature.

There were no mortalities or clinical signs of toxicity. In the FOB, there were treatment-related effects. At 20 000 ppm, rectal temperature was statistically reduced ($P < 0.05$) in comparison with the controls in males and females in week 1, but this was considered an incidental finding and not a consequence of treatment. Motor activity over a 1 h period was similar in control and treated groups. Overall body weight gain in males was reduced by 31% in the 20 000 ppm group, but only by 6% in the 2000 ppm group, and in females by 36%, 30% and 17% in the 20 000 ppm, 2000 ppm and 200 ppm groups, respectively. At the end of treatment, absolute body weight was reduced in males and females by 14% ($P < 0.05$) and 13% (not significant), respectively, at 20 000 ppm compared with controls. Food consumption for males and females was reduced by 41% and 28% when compared with controls during week 1 of treatment, and food conversion ratios over the treatment period were reduced by 24% and 30% in males and females, respectively. Water consumption was increased by 27% and 32% in males and females, respectively. Chemical analysis on blood revealed a slight

Table 8. Summary of selected blood chemistry findings in the 28-day dietary study in rats

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	20	200	2000	20 000	0	20	200	2000	20 000
Cholesterol (mmol/l)	1.61	1.57	2.07	2.42**	2.74***	1.85	2.03	1.93	2.38**	2.67***
ALAT (U/l)	79	75	70	67*	54***	68	69	75	80	63

From Higgs (2000)
ALAT, alanine aminotransferase; U, units; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Bartlett's test)

Table 9. Summary of histopathological findings in liver and kidney in the 28-day dietary study in rats

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	20	200	2000	20 000	0	20	200	2000	20 000
Liver centrilobular hepatocyte hypertrophy										
- Minimal	0	0	1	1	0	0	0	3	2	0
- Slight	0	0	1	4	3	0	0	0	0	5
- Moderate	0	0	0	0	2	0	0	0	0	0
- Total	0	0	2	5	5	0	0	3	2	5
Kidney phloxine tartrazine positive granulation										
- Minimal	4	2	3	0	0	—	—	—	—	—
- Slight	0	2	2	0	1	—	—	—	—	—
- Moderate	1	0	0	3	3	—	—	—	—	—
- Severe	0	0	0	2	1	—	—	—	—	—
- Total	5	4	5	5	5	—	—	—	—	—

From Higgs (2000)

increase in serum cholesterol levels in both sexes at dose levels of 2000 ppm and higher, suggestive of possible impaired liver metabolism, and a slight decrease in alanine aminotransferase activity in males at dose levels of 2000 ppm and above (Table 8).

Absolute liver weights were increased by 24% and 13% in males and females, respectively, and liver weights relative to body weights were increased by 47% in males and by 35% in females.

Microscopic examination of the liver revealed centrilobular hepatocyte hypertrophy in male and female rats of the 200, 2000 and 20 000 ppm groups, but not in the 0 and 20 ppm groups. At 200 ppm, centrilobular hepatocyte hypertrophy was seen in 2 of 5 males and 3 of 5 females, but this finding was not corroborated by any other indices of possible toxicity, and the observation was considered adaptive at this dose level. Additionally, in the kidneys of males, the severity of phloxine tartrazine positive granulation (indicative of granulated lymphocytes) was increased (Table 9).

The NOAEL in the 28-dietary study in rats with fluopicolide was 200 ppm, equal to 17.4 mg/kg bw per day, based on reduction in body weight gain in females, increased levels of cholesterol in both sexes, increases in the absolute and relative liver weights in males, increased incidence and

severity of centrilobular hepatocyte hypertrophy in both sexes and increased incidence and severity of phloxine tartrazine positive granulation in males at 2000 ppm, equal to 174 mg/kg bw per day (Higgs, 2000).

In a 90-day dietary toxicity study, groups of 10 male and 10 female Crl:CD IGS BR Sprague-Dawley rats were administered technical fluopicolide (purity 96.9% and 97.5% in two batch analyses; batch No. 1C990005) in the diet at concentrations of 0, 100, 1400 or 20 000 ppm, equal to 0, 7.4, 109 and 1668 mg/kg bw per day in males and 0, 8.4, 119 and 1673 mg/kg bw per day in females, for at least 13 weeks. An additional group of 10 males and 10 females received either 0 or 20 000 ppm for 13 weeks, after which they were maintained on control diet for a further 4 weeks to investigate the reversibility of any observed effects.

Rats were observed daily for clinical signs, and body weight and food consumption were measured weekly. Water intake was recorded during weeks 4, 8 and 11 of treatment and at the end of the recovery period. An FOB of tests was conducted during week 11 on all rats scheduled to be killed after 13 weeks, and motor activity assessments were conducted during week 12. The FOB assessments were conducted as described above for the 28-day study (Higgs, 2000). Sampling for blood chemistry and haematology assessments was carried out during week 13 of treatment and during week 17 for the recovery groups. Urinalysis was conducted during weeks 12 and 17 for the main and recovery groups, respectively. Eye examinations were conducted before the start and at week 13 of treatment. At necropsy, the weights of selected organs were recorded for all rats. All major organs and tissues were examined microscopically from all main high-dose and control group rats. The liver, kidneys, lungs, adrenals, bone joint and bone marrow from the intermediate groups and caecal sections from all female dose groups were also examined. For recovery group rats, microscopic examinations were conducted on the adrenals, bone joint and bone marrow for both sexes, the caecum from females and the kidneys from males.

Test diets were prepared weekly and analysed for fluopicolide content. The mean results were within the range 94.3–105.9% of nominal (the laboratory's acceptable range being 85–110% of nominal). Homogeneity was satisfactory at all levels; that is, mean values obtained for top, middle and bottom samples were within the acceptable range of 90–110% of nominal, and these mean percentage of nominal values differed by less than 10%. Stability was satisfactory over the time of use of the diet (8 days); in other words, percentage of nominal levels declined by a maximum of 7% over 15 days of storage at room temperature.

There was no treatment-related mortality during the study. Treatment-related clinical signs were recorded only at 20 000 ppm and were characterized by hair loss in both sexes, body soiling and loss of coat condition in males, and a soiled urogenital region in females. The FOB tests (which included grip strength assessment), motor activity and the eye examinations did not provide any evidence of treatment-related effects at any dose level.

Food consumption was lower throughout the study in both sexes at 20 000 ppm and was reduced by 22% in males and 19% in females during week 4. Water intake was increased by 43% during week 4 in females in the 20 000 ppm dose group, but it was not statistically significantly higher in females of this group in week 8 (20%) or week 11 (15%). There was no statistically significant change at other dose levels or in males at any dose level.

At 20 000 ppm, absolute body weight after 13 weeks of treatment was reduced by 30% and 18% in males and females, respectively, when compared with controls. Body weight gain over the course of treatment was reduced by 41% in males and 29% in females. After the recovery period, absolute body weights remained lower at 20 000 ppm by 23% and 18% in males and females, respectively, whereas body weight gains were higher than those of controls by 181% and 117% in males and females, respectively, indicating partial recovery during the 4 weeks without treatment. No body weight changes were observed at the lower dose levels.

Table 10. Summary of selected haematology, blood chemistry and urinalysis findings in weeks 12–13 of the 90-day dietary study in rats

	Males				Females			
	Dietary concentration (ppm)							
	0	100	1400	20 000	0	100	1400	20 000
Haematology								
Haematocrit (l/l)	0.45	0.45	0.43*	0.43*	0.43	0.44	0.43	0.41***
Haemoglobin (g/l)	152	154	145*	143**	151	152	148	137***
MCH (pg)	17.8	18.3	17.5	17.0***	18.8	18.7	18.9	17.1***
MCHC (g/l)	342	341	338	332***	349	350	346	340***
APTT (s)	19.9	18.3	21.2	26.5***	19.8	18.8	18.2	19.0
Blood chemistry								
Cholesterol (mmol/l)	1.63	1.94*						
Total protein (g/l)	64.3	65.7	66.0	70.5**	68.4	67.0	69.5	73.5**
GGT (U/l)	<3	<3	<3	<5	<3	<3	<3	<7
Urinalysis								
Specific gravity	1.022	1.020	1.024	1.017	1.019	1.016	1.011**	1.009**
Urinary volume (ml)	16	19	15	19	16	17	22*	25**

From Mallyon (2000c)
GGT, γ-glutamyl transferase; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Bartlett's test)

Examination of blood indicated small, reversible decreases that never exceeded 9% in haematocrit, haemoglobin, mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in both sexes at 20 000 ppm, whereas activated partial thromboplastin time (APTT) was slightly increased in males only at 20 000 ppm (Table 10). At 1400 ppm, haematocrit and haemoglobin were also slightly, but statistically significantly, reduced. After a 4-week recovery period, no haematological changes were observed compared with controls. No other statistically significant haematological changes were observed at the lower dose levels.

Blood chemistry parameters showed slight reversible increases in cholesterol, total protein and activity of γ-glutamyl transferase (GGT) in both sexes at 20 000 ppm (Table 10). At 1400 ppm, a small, reversible increase in cholesterol was also observed in males. No toxicologically significant findings were observed at 100 ppm.

In urinalysis, a slight increase in the number of epithelial cells in the urinary sediment was observed in males at 20 000 ppm. A slight decrease in specific gravity was observed in females at 20 000 and 1400 ppm that was associated with increases in urinary volume (Table 10). No statistically significant changes were observed at the lower dose levels.

The 20 000 ppm group rats showed an increased absolute liver weight of 22% in females and increased liver weights relative to body weight of 50% in males and 49% in females. Absolute spleen weights were decreased by 45% and 40% in males and females, respectively, and spleen weights relative to body weight were decreased by 24% and 29% in males and females, respectively. Kidney weights relative to body weight were increased by 15% in males.

At 1400 ppm, absolute organ weights were not significantly altered, but liver weights relative to body weight were increased by 15% in males, relative spleen weights were decreased by 19% in females and relative kidney weights were increased by 11% in males. There were no effects on organ

Table 11. Summary of the main histopathological changes in the adrenal glands, liver and bone in the 90-day dietary study in rats

Parameter	Incidence (no. of rats affected/total no. of rats examined)							
	Males				Females			
	Dietary concentration (ppm)							
	0	100	1400	20 000	0	100	1400	20 000
Week 13								
Adrenal – hypertrophy zona glomerulosa								
- Minimal	1/10	0/10	0/10	0/10	1/10	1/10	3/10	3/10
- Slight	0/10	0/10	0/10	7/10	0/10	0/10	0/10	7/10
- Total	1/10	0/10	0/10	7/10	1/10	1/10	3/10	10/10
Liver – centrilobular hypertrophy								
- Minimal	0/10	0/10	3/10	1/10	0/10	0/10	0/10	4/10
- Slight	0/10	0/10	5/10	0/10	0/10	0/10	0/10	4/10
- Moderate	0/10	0/10	0/10	8/10	0/10	0/10	0/10	0/10
- Total	0/10	0/10	8/10	9/10	0/10	0/10	0/10	8/10
Bone joint – trabecular hyperostosis	0/10	0/10	0/10	7/10	3/10	1/10	8/10	9/10
Bone marrow – decreased cellularity	0/10	1/10	0/10	7/10	3/10	0/10	0/10	9/10
Week 17								
Adrenal – hypertrophy zona glomerulosa								
- Minimal	0/10	—	—	8/10	1/10	—	—	4/10
- Slight	0/10	—	—	0/10	0/10	—	—	1/10
- Total	0/10	—	—	8/10	1/10	—	—	5/10
Bone marrow – decreased cellularity	0/10	—	—	3/10	4/10	—	—	5/10

From Mallyon (2000c)

weight in either sex at 100 ppm. After 4 weeks of recovery, there was a trend for a reversal of all the effects on the liver and spleen weights of males and females and the kidney weights of males.

Gross examination at necropsy revealed speckled appearance of the kidneys in 4 of 10 males at 20 000 ppm and in 3 of 10 males at 1400 ppm. After 4 weeks of recovery, this effect was reduced.

Microscopic examination of organs and tissues revealed dose-related changes in the liver and kidneys as well as in the adrenals, bone joint and bone marrow (Tables 11 and 12).

Liver changes consisted of minimal to moderate centrilobular hepatocyte hypertrophy in both sexes at 20 000 ppm and minimal to slight hypertrophy of centrilobular hepatocytes in most of the male rats only at 1400 ppm. There was complete recovery by week 17 at 20 000 ppm after the recovery period (Table 11).

Table 12. Summary of the main histopathological changes in the kidney in the 90-day dietary study in male rats

Parameter	Incidence (no. of rats affected/total no. of rats examined)			
	Dietary concentration (ppm)			
	0	100	1400	20 000
Week 13				
Accumulation of hyaline droplets				
- Minimal	1/10	0/10	0/10	0/10
- Slight	5/10	0/10	0/10	0/10
- Moderate	0/10	2/10	1/10	1/10
- Severe	0/10	0/10	9/10	7/10
- Total	6/10	2/10	10/10	8/10
Kidneys – single cell death – proximal tubule epithelium				
- Minimal	5/10	0/10	0/10	1/10
- Slight	0/10	2/10	1/10	1/10
- Moderate	0/10	0/10	9/10	6/10
- Total	5/10	2/10	10/10	8/10
Kidneys – foci of basophilic tubules				
- Minimal	6/10	3/10	0/10	1/10
- Slight	0/10	0/10	4/10	7/10
- Moderate	0/10	0/10	6/10	0/10
- Total	6/10	6/10	10/10	8/10
Kidneys – granular casts				
- Minimal	0/10	0/10	1/10	1/10
- Slight	0/10	0/10	0	3/10
- Total	0/10	0/10	1/10	4/10
Week 17				
Accumulation of hyaline droplets				
- Minimal	1/10	—	—	0/10
- Slight	4/10	—	—	0/10
- Moderate	2/10	—	—	1/10
- Total	7/10	—	—	8/10
Kidneys – single-cell death – proximal tubule epithelium		—	—	
- Minimal	4/10	—	—	2/10
- Slight	2/10	—	—	2/10
- Total	6/10	—	—	4/10
Kidneys – foci of basophilic tubules		—	—	
- Minimal	7/10	—	—	3/10
- Slight	0/10	—	—	5/10
- Total	7/10	—	—	8/10
Kidneys – granular casts				
- Not present	10/10	—	—	10/10

From Mallyon (2000c)

Adrenal changes consisting of a dose-related increase in the incidence and severity of minimal to slight hypertrophy of the zona glomerulosa was observed at 20 000 ppm in both sexes and at 1400 ppm in females only. A distinct reduction in the severity was observed at the end of the off-dose period (Table 11).

Trabecular hyperosteosis of the bone joint was observed in males and females at 20 000 ppm and in females at 1400 ppm. Reduced cellularity of the bone marrow was observed in 3 of 10 males at 20 000 ppm, compared with 0 of 10 in the controls, but incidences in females were essentially the same, with 4 of 10 in controls and 5 of 10 in the 20 000 ppm group (Table 11). The microscopic changes observed in bones and adrenals in this study were not observed again in subsequent chronic toxicity studies in rats.

Kidney effects consisted of an increase, in males only, in the severity and incidence of hyaline droplet accumulation in the proximal tubules, single-cell necrosis in the proximal tubule epithelium, foci of basophilic (regenerating) tubules and granular casts at 20 000 and 1400 ppm. After the 4-week recovery period, partial recovery was observed for all the kidney effects (Table 12).

The NOAEL in the 90-day dietary study in rats was 100 ppm, equal to 7.4 mg/kg bw per day, based on treatment-related reduction in haemoglobin and haematocrit values in male rats, increased cholesterol in male rats, increased urine volume and specific gravity in females, increases in relative liver and kidney weight in male rats and in relative spleen weight in females, and histopathological changes in the liver and kidneys at 1400 ppm, equal to 109 mg/kg bw per day (Mallyon, 2000c).

Dogs

In a 28-day toxicity study in dogs, groups of two male and two female Beagle dogs were administered fluopicolide (purity 96.9%; batch mixture PP/241024/2 and PP/241067/1) by gavage at dose levels of 0, 10, 100 and 1000 mg/kg bw per day for at least 28 consecutive days. Fluopicolide was administered in a vehicle of 1% w/v methyl cellulose in distilled water at a constant volume of 5 ml/kg bw. Controls received the vehicle alone. Dogs were observed for clinical signs daily; food consumption was measured daily, and body weight was recorded weekly. A detailed clinical and eye examination was carried out on all dogs before treatment and at the end of the treatment period. Haematology and blood chemistry investigations were conducted before treatment (days −14 and −1), during treatment (day 14) and at the end (day 29) of the treatment period. Urinary parameters were measured at termination. At necropsy, the weights of selected organs were recorded, and a range of tissues was prepared for microscopic examination.

The homogeneity and stability of the fluopicolide preparations were analysed at all dose levels from the trial mix samples stored at room temperature for 0, 1, 2 and 4 days. The study mix samples from days 1 and 22 were analysed at all dose levels. The mean results for the test suspension samples analysed were within the range 82.9–102.8% of nominal. Homogeneity in the trial mix was within the acceptable range of 80–120% of nominal, and standard deviation values were less than 10%. Reanalysis of the trial mix after storage at 4 ºC for 0, 1, 2 and 4 days indicated that the suspensions were stable over 4 days at this temperature.

During the treatment period, the dogs were fed with 400 g of pelleted dog diet between 1 and 2 h after treatment. Body weights at the start of treatment ranged from 10.5 to 11.9 kg for males and from 8.3 to 9.8 kg for females.

There were no mortalities, and no clinical signs of toxicity were observed during the study. Body weights and food consumption were not affected by the treatment. The findings of eye examination, haematology and urinalysis parameters did not reveal any treatment-related intergroup differences. Blood chemistry analysis showed a slight increase in cholesterol concentration on days 14 (176%) and 29 (220%) in one male. This dog was also noted to have an enlarged liver at necropsy and increases in absolute and relative (to body weight) liver weight of 34% and 44%, respectively. No

Table 13. Summary of the organ weight changes in the liver in the 90-day oral study in dogs

Organ weight at week 13	Males				Females			
	Dose level (mg/kg bw per day)							
	0	5	70	1000	0	5	70	1000
Absolute liver weight (g)	368.1	351.1	350.6	438.8*	300.1	322.3	318.6	396.4
Relative liver weight (%)	2.9	2.7	2.9	3.7*	2.8	2.9	2.9	4.0*

From Mallyon (2000e)
* $P < 0.05$

treatment-related microscopic changes were observed. There were no treatment-related effects in males at 100 or 10 mg/kg bw per day or in any treated females.

The NOAEL in the 28-day oral toxicity study in dogs was 100 mg/kg bw per day based on increased blood cholesterol concentrations associated with increased absolute and relative liver weights in 1 of 2 males at 1000 mg/kg bw per day (Mallyon, 2000d).

In a 90-day oral toxicity study in dogs, groups of four male and four female outbred Beagle dogs were administered fluopicolide (purity 97.7%; batch mixture PP/241024/2 and PP/241067/1) by gavage at dose levels of 0, 5, 70 or 1000 mg/kg bw per day for at least 13 weeks. Fluopicolide was administered in a vehicle of 1% w/v methyl cellulose in distilled water at a constant volume of 5 ml/kg bw. Controls received the vehicle alone. Dogs were observed daily for clinical signs of toxicity; food consumption was measured daily, and body weight was recorded weekly. A detailed clinical examination and ophthalmoscopy were carried out on all dogs before treatment and at the end of the treatment period. Haematology and blood chemistry investigations were conducted before the start (days −14 and −1), during (week 7) and at the end of the treatment period. Urinalysis parameters were measured before treatment, during week 6 and at termination. At necropsy, the weights of selected organs were recorded, and a range of tissues was prepared for microscopic examination.

Test diets were prepared weekly and analysed for fluopicolide content. The mean results for the test diet samples analysed were within the range 94.3–105.9% of nominal. Homogeneity was shown to be satisfactory at all levels; that is, mean values obtained for top, middle and bottom samples were within the acceptable range 90–110% of nominal, and these mean percentage of nominal values differed by less than 10%. Stability was satisfactory over the time of use of the diet (8 days); in other words, percentage of nominal levels declined by a maximum of 7% over 15 days of storage at room temperature.

There were no mortalities, and no clinical signs of toxicity were observed. There were no treatment-related effects on body weight, feed intake, the eyes, haematology, blood chemistry, urinalysis, macroscopic pathology or the microscopic parameters investigated.

At 1000 mg/kg bw per day, absolute liver weight was not statistically significantly increased in females, but there was a statistically significant increase in males. Liver weight relative to body weight was statistically significantly increased by 50% and 49% in males and females at this dose level. Organ weights were unaffected at 70 and 5 mg/kg bw per day, respectively (Table 13).

The NOAEL in the 90-day dietary study in dogs was 70 mg/kg bw per day, based on increased relative liver weight at 1000 mg/kg bw per day in males (Mallyon, 2000e).

In a 52-week oral toxicity study in dogs, groups of five male and five female Beagle dogs were administered fluopicolide (purity 95.9%; batch No. OP2050046) by oral gavage as a suspension in 1% aqueous methyl cellulose solution at dose levels of 0, 70, 300 or 1000 mg/kg bw per day for

1 year. The dogs were checked for mortality and morbidity at least twice daily and for clinical signs of toxicity once a day. A detailed clinical examination was performed once before the first treatment and once weekly throughout the treatment period. Body weights were recorded at least once before the start of the study, on the first day of treatment and then at weekly intervals. Food consumption was recorded daily. Ophthalmoscopic examinations were performed once before treatment and during weeks 12, 25 and 51. Haematological, blood chemical and urinalysis investigations were performed before the treatment period and during weeks 13, 26 and 51. On completion of treatment, the dogs were killed. All dogs were subjected to a complete gross postmortem examination. Selected organs were weighed, and specified tissues were preserved. Selected tissues from all dogs were examined microscopically.

There was no treatment-related mortality during the study. One female of the 300 mg/kg bw per day dose group died, but no specific cause of death was identified. Review of the clinical signs before death and macroscopic and microscopic postmortem examinations suggested accidental aspiration of stomach contents into the lungs after regurgitation as a possible cause of death. Clinical signs observed were slightly increased incidences of regurgitation and excessive salivation in dogs at dose levels of 300 and 1000 mg/kg bw per day. The effect, however, was possibly due to the administration of the viscous dose preparation (200 mg/ml), rather than a pharmacological effect of the compound itself.

At 1000 mg/kg bw per day, there was impairment of body weight gain in males, and no body weight gain was observed at 52 weeks compared with the start of the study. Body weights and body weight gains in males given 70 and 300 mg/kg bw per day and in all treated females were similar to those of control dogs.

Food consumption was normal. Haematology and urinalysis parameters did not reveal any treatment-related changes. Blood chemistry showed significant increases in plasma cholesterol levels in females at 1000 mg/kg bw per day in week 51 (+42%, $P < 0.05$).

Organ weights were not significantly affected by treatment. Postmortem, the liver was enlarged in 3 of 5 males given 1000 mg/kg bw per day and in 1 of 5 males and 1 of 5 females given 300 mg/kg bw per day. The kidneys were enlarged in 1 of 5 males given 300 mg/kg bw per day; however, considering the absence of a dose–response, this observation is not considered to be treatment related. Microscopic examination did not reveal any changes in organs and tissues, and the gross observations in the absence of significant organ weight change are considered to be of uncertain toxicological significance.

The NOAEL in the 1-year oral toxicity study was 300 mg/kg bw per day, based on impaired body weight gain in males and slightly increased cholesterol levels in females at 1000 mg/kg bw per day (Chevalier, 2002).

(b) Dermal application

Rats

In a 28-day dermal toxicity study, 10 male and 10 female Wistar rats were administered fluopicolide (purity 97.7%; batch No. 2050190/PP241024/2) at dose levels of 0, 100, 250, 500 and 1000 mg/kg bw per day applied under a semiocclusive dressing to intact shaven dorsal skin on 5 days/week for 4 weeks. The dose was based on the body weight of each rat on days 0, 7, 14, 21 and 28. During the study, the rats were evaluated for the effect of the test compound on body weight, food consumption, clinical signs, the eyes, blood chemistry and haematology. Gross postmortem evaluations were performed on all rats, and microscopic assessment of selected organs and tissues was conducted on the control and high dose groups.

There were no treatment-related deaths during the study. There were no treatment-related effects on body weight or food consumption. There were no treatment-related effects on blood chem-

istry values or haematological parameters. There were no treatment-related effects on organ weights. There were no treatment-related gross pathological or histopathological changes in the organs and tissues.

The NOAEL in the 28-day dermal toxicity study in rats was 1000 mg/kg bw per day, based on the absence of toxicity at the highest dose investigated (Eigenberg & Stuart, 2003).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female C57BL/6 mice were administered fluopicolide (purity 95.9%; batch No. OP2050046) in the diet at concentrations of 0, 50, 400 or 3200 ppm, equal to 0, 7.9, 64.5 and 551.6 mg/kg bw per day for males and 0, 11.5, 91.9 and 772.9 mg/kg bw per day for females, for 78 weeks. For the evaluation of chronic toxicity, additional satellite groups of 10 of each sex were treated and killed after 1 year.

Throughout the study, mice were examined daily for clinical signs of toxicity and for mortality, and careful examination was carried out before the beginning of the treatment period and weekly thereafter to assess possible neurotoxic effects. Palpation for masses was carried out every 4 weeks from weeks 4 to 52 and every 2 weeks thereafter. Body weight and feed consumption were measured at weekly intervals during the first 13 weeks of the study, every 4 weeks until weeks 31/32 and every 2 weeks thereafter. Achieved dosages were calculated. Before the satellite group, mice were killed in week 52, and blood was taken for the measurement of the activities of the liver-associated enzymes aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase. At the end of the appropriate scheduled treatment period (week 52 or 78), mice were killed and submitted to a macroscopic postmortem examination. A complete range of organs and any masses or macroscopic lesions were sampled. A microscopic examination was performed on all long-duration groups of mice as well as on the liver and on macroscopic abnormalities from the satellite groups of mice.

Survival rates were compared using the chi-squared test. The numbers of neoplasms (per group and per organ) were compared by Peto's test.

Before the start of the treatment period, the dietary admixtures were analysed for the concentration, homogeneity and stability of fluopicolide. High-performance liquid chromatography (HPLC) analysis of the test substance carried out every 6 months confirmed that the purity remained the same throughout the treatment period. The results of the analyses demonstrated the satisfactory homogeneity of each dietary admixture analysed during the study. Furthermore, there was a good correspondence between the nominal and the measured concentrations of the test item in the diet. Stability of the formulation was satisfactory for a minimum of 10 days.

Survival statistics as well as the factors contributing to mortality were similar in the control and treated groups. Survival rates over 78 weeks were 82%, 88%, 90% and 88% in males and 90%, 82%, 92% and 82% in females at the corresponding 0, 50, 400 and 3200 ppm dose groups. The incidence, nature and onset of the clinical signs were similar in the control and treated groups. No signs of neurotoxicity were observed during the study. The frequency, time of onset and size of the few palpable masses recorded were similar in the control and treated groups.

The body weight and the body weight gain of the 3200 ppm dose group were statistically significantly decreased (Table 14). This effect was associated with a slight reduction in food consumption (−7% in males and −8% in females) throughout the study. Food consumption and body weights of treated mice at 50 or 400 ppm were similar to those of controls.

Blood chemistry parameters investigated in satellite group mice did not generally reveal any differences in liver-associated enzyme activities between treated and control mice. However, there

Table 14. Group mean body weights and body weight changes throughout the treatment period in the 78-week dietary study in mice

Parameter	Males				Females			
	Dietary concentration (ppm)							
	0	50	400	3200	0	50	400	3200
Body weight (g)								
Week 2	23.9	23.9	24.0	23.1**	20.3	20.4	20.3	19.2**
Week 13	29.8	29.9	29.0*	26.9*	24.5	24.5	24.8	22.8**
Week 26	34.9	34.8	33.8	29.1**	28.1	28.8	28.7	24.5**
Week 52	40.8	41.3	39.0	31.9**	33.3	34.5	34.1	26.7**
Week 78	41.4	43.5	42.0	33.3**	34.7	36.1	36.3	29.2**
Body weight change (g)								
Week 13 versus week 2	5.9	6.0	5.0**	3.9**	4.2	4.1	4.5	3.7*
Week 26 versus week 13	5.1	4.9	4.8	2.2**	3.6	4.2	3.9	1.7**
Week 52 versus week 26	5.8	6.4	5.3	2.8**	5.2	5.7	5.4	2.2**
Week 78 versus week 1	18.3	20.5	18.8	10.0**	15.3	16.8	16.9	9.9**

From Chevalier (2003)
* $P < 0.05$; ** $P < 0.01$

Table 15. Group mean liver enzyme activities at 53 weeks in the 78-week dietary study in mice (n = 10 per group)

Enzyme	Enzyme activity (IU/l)							
	Males				Females			
	Dietary concentration (ppm)							
	0	50	400	3200	0	50	400	3200
Alkaline phosphatase	129	122	114*	135	180	170	183	564**
Aspartate aminotransferase	80	61	62	65	75	101	115	194
Alanine aminotransferase	28	24	40	64	45	29	34	145

From Chevalier (2003)
IU, international units; * $P < 0.05$; ** $P < 0.01$

was a notable increase in the mean alkaline phosphatase activity in females at 3200 ppm accompanied by higher, but statistically non-significant, alanine and aspartate aminotransferase activities. These findings appeared to be due to very large changes in 2 of 10 mice (Table 15).

Organ weights at postmortem examination revealed increased absolute and relative liver weights at dose levels of 400 and 3200 ppm at the end of both the 52-week and 78-week treatment periods (Table 16). These changes were associated with hepatocellular hypertrophy in these mice.

At necropsy of the mice after 52 weeks, liver enlargement was observed in males of the 400 and 3200 ppm groups, and liver masses and nodules were observed in females treated at 3200 ppm.

Table 16. Summary of liver weight changes in mice after 52 weeks and 78 weeks of treatment compared with controls

Parameter	Males			Females		
	Dietary concentration (ppm)					
	50	400	3200	50	400	3200
After 52 weeks of treatment (n = 10)						
Absolute liver weight (%)	+8	+30**	+35**	−5	+4	+50*
Relative liver weight (%)	+8	+15**	+63**	0	+10	+99**
After 78 weeks of treatment (n = 50)						
Absolute liver weight (%)	+14	+18**	+46**	−1	+33**	+56**
Relative liver weight (%)	+9	+15**	+79**	−5	+28	+81**

From Chevalier (2003)
* $P < 0.05$; ** $P < 0.01$

Table 17. Summary of the incidence of mice bearing liver neoplasms and non-neoplastic lesions (hepatocellular hypertrophy and altered cell foci) after 78 weeks in the carcinogenicity study in mice

Finding	Incidence[a]							
	Males				Females			
	Dietary concentration (ppm)							
	0	50	400	3200	0	50	400	3200
Hepatocellular hypertrophy	0	0	20	49	0	0	41	46
Total foci of altered hepatocytes	1	8	5	18	1	3	4	25
Hepatocellular adenoma	5	0	5	11	1	2	0	16
Hepatocellular carcinoma	3	1	0	2	0	0	2	0
Total hepatocellular adenoma plus carcinoma	8	1	5	13	1	2	2	16

From Chevalier (2003)
[a] Number of mice with neoplasms or lesions out of 50 mice examined.

After 78 weeks, there was a marked increase of liver enlargement at 3200 ppm, and the numbers of mice bearing liver masses and nodules in the 400 and 3200 ppm groups were increased.

Microscopic examination at both 52 and 78 weeks showed a dose-related hepatocellular hypertrophy at 400 and 3200 ppm, a higher incidence of altered cell foci at 3200 ppm and a markedly and statistically significantly higher incidence of hepatocellular adenoma at 3200 ppm.

After 52 weeks, dose-related incidences of centrilobular hepatocellular hypertrophy were observed at 400 ppm (5/10 males and 6/10 females) and 3200 ppm (10/10 males and 9/10 females). Hepatocellular adenomas were found in 1 of 10 females given 400 ppm and in 3 of 10 females given 3200 ppm. The higher incidence of hepatocellular adenoma at 3200 ppm ($P < 0.036$) was considered to be treatment related.

After 78 weeks, the overall numbers of mice with neoplasms, the number of mice with more than one primary neoplasm and the number of mice with benign and malignant tumours were similar in all groups. However, higher incidences of hepatocellular adenomas were noted in the males and females given 3200 ppm (Table 17). The incidence and time of onset of the hepatocellular neoplastic lesions in the other treated groups (50 ppm and 400 ppm) were similar to those of the controls. There was no evidence for a tendency for adenomas to progress to carcinomas.

The differential diagnosis of hepatocellular adenoma and hepatocellular carcinoma was as follows. Adenoma was diagnosed when cells resembling relatively normal hepatocytes formed discrete nodules that significantly compressed the adjacent parenchyma and sometimes bulged above the surface. The diagnosis of hepatocellular carcinoma was made when the liver plates were more than one layer thick, irregular and composed of well to moderately differentiated hepatocytes. The lesions were either solid or trabecular and showed great variability in cell and nuclear size. Large cells with large hyperchromatic nuclei were commonly present. Many such cells seemed to be undergoing necrosis.

The NOAEL in the 78-week dietary study in mice was 50 ppm, equal to 7.9 mg/kg bw per day, based on increased liver weights, enlarged liver, masses and nodules in the liver, and hepatocellular hypertrophy at 400 ppm, equal to 64.5 mg/kg bw per day. Fluopicolide was tumorigenic in mice and induced hepatocellular adenomas in male and female mice at a dose level of 3200 ppm, equal to 552 mg/kg bw per day in male mice and 773 mg/kg bw per day in female mice (Chevalier, 2003).

Rats

In a chronic toxicity and carcinogenicity study, groups of 90 male and 90 female Crl:CD(SD) IGS BR rats were administered fluopicolide (purity 95.9%; batch No. OP2050046) in the diet at concentrations of 0, 50, 200, 750 or 2500 ppm. Each group was divided into three segments: 1) subgroups of 50 of each sex that were treated for up to 104 weeks, during which the doses were equal to 0, 2.1, 8.4, 31.5 and 109.4 mg/kg bw per day for males and 0, 2.8, 10.8, 41.0 and 142.2 mg/kg bw per day for females; 2) subgroups of 20 of each sex that were killed after completion of 52 weeks of treatment (the toxicity phase of the study); and 3) subgroups of 10 of each sex that were treated for 52 weeks, followed by a 13-week period without treatment to assess the reversibility of any treatment-related findings (the recovery phase of the study). The doses during the 52-week treatment period were equal to 0, 2.5, 9.8, 37.0 and 125.5 mg/kg bw per day for males and 0, 3.3, 12.9, 48.7 and 163.6 mg/kg bw per day for females.

During the study, examination of the rats was undertaken for their clinical condition, palpation, body weight, food consumption, ophthalmoscopy, haematology, blood chemistry, urinalysis, organ weight, and macroscopic and microscopic pathology.

All formulations were shown to be homogeneous in the diet and stable at ambient temperature for up to 22 days. The mean concentrations of fluopicolide assayed in weeks 1, 13, 26, 39, 52, 65, 79, 91 and 103 were within 7% of intended specifications and were therefore considered satisfactory.

There was no treatment-related effect on survival. Cumulative mortality in the carcinogenicity-phase rats at 105 weeks was 37, 32, 32, 29 and 31 in males and 39, 45, 37, 36 and 35 in females at the corresponding dietary concentrations of 0, 50, 200, 750 and 2500 ppm. Clinical signs throughout the majority of the treatment period included increased incidences of yellow perigenital staining, brown staining on the dorsal body surface and/or brown staining of the pinnae in female rats at dose levels of 750 or 2500 ppm. These signs tended to resolve during the recovery period. There were no treatment-related signs among rats receiving 50 or 200 ppm. Ophthalmoscopic examinations during weeks 51, 78 and 104 of the study did not reveal any treatment-related findings.

Males and females receiving the 2500 ppm diet consumed less food than the controls in the first week of treatment (−13% and −7% for the toxicity-phase males and females, respectively, and −20% and −7% for the carcinogenicity-phase males and females, respectively). Thereafter, females receiving the 2500 ppm diet tended to consume slightly less feed than the controls during most of the study; consequently, the total feed intake of these rats was slightly reduced (around −7%).

In the recovery phase of the study, all groups of rats, including controls, consumed similar amounts of feed, with the exception of females that had received the 2500 ppm diet, for which the feed intake remained slightly reduced (−11% when compared with the controls).

Body weight was markedly reduced in both sexes at 2500 ppm during the first week of treatment; this was associated with low feed intake and increased feed scatter (Table 18). Subsequent

Table 18. Group mean body weight gains

Time period	Body weight gain (g)									
	Group/sex									
	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
	Dietary concentration (ppm)									
	0	50	200	750	2500	0	50	200	750	2500
Toxicity phase										
Weeks 0–1	57	56	53	50**	35**	28	32*	18**	17**	13**
Weeks 0–52	556	559	559	554	527	297	273	273	257	222**
Carcinogenicity phase										
Weeks 0–1	52	53	56*	49	35**	25	23	20**	7**	18**
Weeks 0–104	623	629	665	602	555	390	346	378	396	322

From Cooper (2003)
* $P < 0.05$; ** $P < 0.01$

Tables 19. Group mean haematological changes on week 104

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	50	200	750	2500	0	50	200	750	2500
Haematocrit (l/l)	0.425	0.425	0.439	0.420	0.409	0.404	0.408	0.415	0.399	0.382*
Haemoglobin (g/dl)	14.4	14.4	14.9	14.1	13.6	14.0	14.2	14.4	13.8	13.1*
RBC ($\times 10^{12}$/l)	7.68	7.71	7.98	7.38	7.68	6.97	7.09	7.14	6.87	6.62
MCH (pg)	18.8	18.8	18.7	19.2	17.7**	20.1	20.1	20.2	20.2	20.0
MCHC (g/dl)	33.8	33.9	33.9	33.4	33.2**	34.6	34.8	34.7	34.7	34.4
MCV (fl)	55.6	55.3	55.0	57.5	53.2*	58.1	57.7	58.2	58.1	58.1

From Cooper (2003)
RBC, red blood cells; * $P < 0.05$; ** $P < 0.01$ (Fisher's exact test)

weight gain by rats receiving 2500 ppm tended to be lower than that of the controls, although the difference was less than was seen during the first 2 weeks of treatment. Transient reduction in body weight gain was also observed in females at dietary concentrations of 200 ppm and above and in males at 750 ppm and higher. The subsequent weight gain of females receiving 200 or 750 ppm was similar to that of the controls.

Haematological changes during the study were observed predominantly in the 2500 ppm dose group, with males being more affected than females (Table 19). At 2500 ppm, low haemoglobin concentrations were recorded for males and females throughout most of the treatment period. Occasionally associated with this was a reduction of haematocrit in males and in females and low erythrocyte counts in females. As a consequence of these changes, MCH was consistently low in males, and MCHC and mean corpuscular volume (MCV) were also low at most measurement times in these rats. No toxicologically significant haematological changes were observed in rats at 50, 200 or 750 ppm.

Blood chemistry analysis of the 2500 ppm group rats showed significantly increased total protein concentrations in males and females and reduced albumin to globulin ratios up to week 52 in

Table 20. Group mean blood chemistry changes on week 52 or 104

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	50	200	750	2500	0	50	200	750	2500
Week 52										
Total protein (g/l)	69	67	68	69	71*	77	78	79	77	82*
A/G ratio	0.97	1.01	0.96	0.95	0.93	1.12	1.16	1.08	1.12	1.01**
Week 104										
Creatinine (µmol/l)	48	50	50	52	58*	49	48	47	45	49
Cholesterol (mmol/l)	3.34	3.24	4.08	4.49	4.39	3.53	3.52	3.20	3.61	4.15
Calcium (mmol/l)	2.85	2.88	2.87	2.95*	3.07**	2.77	2.83	2.87*	2.96**	2.95**
Potassium (mmol/l)	3.5	3.6	3.6	4.1**	4.0**	3.3	3.1	3.2	3.6	3.9*

From Cooper (2003)
A/G, albumin to globulin ratio; * $P < 0.05$; ** $P < 0.01$ (Fisher's exact test)

females (Table 20). High creatinine concentrations in males and a trend towards marginally increased total cholesterol concentrations in males and females were seen, whereas high potassium and calcium concentrations were observed in both sexes in weeks 52 and 104. None of these changes was present at the end of the recovery period. No toxicologically relevant changes were observed in rats at 50 and 200 ppm. At 750 ppm, blood chemistry changes in the plasma comprised marginally increased total protein concentration in males associated with low albumin to globulin ratio, marginally increased cholesterol concentrations in males and occasionally increased potassium and calcium concentrations in males and females at week 52 or week 104. These changes were not observed at the end of the recovery period.

At week 104, absolute weights of kidneys were significantly increased in male rats of the 200, 750 and 2500 ppm groups by 109%, 109% and 122%, respectively, but not in female rats of any group. Absolute weights of livers were significantly increased by 122% in male rats of the 2500 ppm group. No changes in organ weights observed at week 52 remained after the 13-week recovery period. No treatment-related organ weight changes were observed at 50 ppm.

Gross examination postmortem did not reveal any treatment-related macroscopic changes after 52 weeks of treatment at 750 and 2500 ppm. After 104 weeks of treatment, there were, in comparison with the controls, slightly higher incidences of enlarged kidneys in males at 2500 ppm.

Microscopic examination after 52 weeks of treatment indicated a dose-related increased incidence and severity (slight to moderate) of centrilobular hepatocyte hypertrophy and an increased incidence and/or severity of cortical tubular basophilia in the kidneys in males at 2500 and 750 ppm (Table 21). At 2500 ppm, the change in the kidneys was associated with increased incidences of hyaline droplets within the cortical tubules, hyaline tubular casts and granular medullary casts. After completion of the 13-week recovery period, there remained a slight increase in the severity of cortical tubular basophilia in the kidneys of the males, but all other changes had fully resolved. There were no treatment-related histopathological findings after 52 weeks of treatment at dose levels of 200 ppm and below.

In the carcinogenicity study, no treatment-related neoplastic findings were observed. At 2500 or 750 ppm, microscopic examination indicated changes in the liver, kidneys, pancreas, prostate and thyroid (Table 22). In the liver, there was an increased incidence of centrilobular hepatocyte

Table 21. Microscopic findings in liver and kidneys of males after 52 weeks of treatment

Findings		Dietary concentration (ppm)				
		0	50	200	750	2500
Liver	Centrilobular hypertrophy	0/20	0/20	0/15	14/20***	19/20***
Kidneys	Cortical tubular basophilia	7/20	10/20	9/15	20/20***	20/20***
	Cortical tubules with hyaline droplets	0/20	1/20	0/15	2/20	13/20***
	Granular casts	0/20	0/20	0/15	0/20	7/20**
	Hyaline tubular casts	6/20	5/20	4/15	8/20	17/20**

From Cooper (2003)
** $P < 0.01$; *** $P < 0.001$ (Fisher's exact test)

Table 22. Summary of selected microscopic findings after 104 weeks of treatment

Finding		Incidence (no. observed out of 60 rats examined)[a]									
		Males					Females				
		Dietary concentration (ppm)									
		0	50	200	750	2500	0	50	200	750	2500
Liver	Centrilobular hypertrophy	0	0	2	9**	18***	0	0	0	0	0
	Cystic degeneration	13	17	18	23	32***	0	1	0	0	0
	Eosinophilic foci	13	17	15	19	25*	4	6	5	11	15*
Kidneys	Tubular casts	24	30	32	32	45***	23	17	20	24	25
	Cortical tubular dilatation	10	9	9	8	27**	8	7	9	12	14
	Cortical tubules with hyaline droplets	3	3	3	4	14**	1	2	0	1	0
	Hyperplasia papilla	14	14	18	20	11	36	28	32	47*	43
	Mineralization papilla	0	1	2	2	12***	4	3	2	0	3
Prostrate	Acinar cell atrophy	19	12/35	7/34	6/31	31*					
	Reduced colloid	8	3/35	7/34	3/31	17					

From Cooper (2003)
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Fisher's exact test)
[a] Except where otherwise noted for prostate.

hypertrophy and an increased incidence or severity of cystic degeneration and foci of alteration in males and an increased incidence of eosinophilic foci of alteration in females. An increased incidence of cystic follicular cell hyperplasia in the thyroids of males was also observed.

At 2500 ppm in both sexes, but males in particular, there were increased incidences of hyaline droplets in the renal cortical tubules, cortical tubular dilatation and tubular casts, as well as indications of proliferative changes, comprising cortical tubular basophilia. Hyperplasia of the papillary epithelium at 2500 and 750 ppm was present at an increased incidence and severity in females, and this was usually associated with mineralization of the papillary/pelvic epithelium (2500 ppm only).

An increased incidence of acinar atrophy, often associated with reduced colloid, was present in the prostate of high-dose males in the carcinogenicity-segment rats only. No treatment-related changes were observed at dose levels of 200 ppm and below.

The NOAEL in the 2-year chronic toxicity and carcinogenicity study in rats was 200 ppm, equal to 8.4 mg/kg bw per day, based on increased centrilobular hypertrophy of the liver at 750 ppm, equal to 32 mg/kg bw per day (Cooper, 2003).

2.4 Genotoxicity

Fluopicolide was tested for genotoxicity in a range of assays, both in vitro and in vivo (Table 23). All study references are listed in Table 23. In one of five bacterial reverse mutation assays, there was an increase in the number of revertant colonies in one strain (*Salmonella typhimurium* strain TA98) in the standard plate test and in *S. typhimurium* strains TA98 and TA1537 in the presence of S9 and TA1535 in the absence of S9 in the preincubation test. The significant responses were at dose levels above the solubility of the test material. Precipitation was first recorded at 500 µg/plate, whereas the maximum response from the most sensitive strain, TA98, was at about 4000–5000 µg/plate, suggesting that the effect could be due to a contaminant. Four additional assays with different batches of fluopicolide were performed in a different laboratory. There was no evidence of mutagenic activity in the four additional bacterial reverse mutation assays performed with the same *S. typhimurium* and *Escherichia coli* strains over the same dose range, but with different batches. These batches included batch No. OP2050046 (purity 95.9%), which was used in the carcinogenicity study in mice. The same mixture of batches (PP/241024/2 + PP/241067/1) as induced significant responses in bacteria (Stammberger, 2000) was tested for gene mutation, inducing activity at the *hprt* locus in Chinese lung V79 cells. There was no significant increase in the proportion of mutant colonies at any dose level. The maximum concentrations that did not induce excessive toxicity in these cells were 50 µg/ml in the absence of S9 and 38 µg/ml in the presence of S9. The next higher concentrations tested reduced survival to 1.5% (75 µg/ml in the absence of S9) and 4.3% (50 µg/ml in the presence of S9).

Table 23. Results of studies of genotoxicity with fluopicolide

End-point	Test object	Concentration/dose (LED/HID)	Batch; purity (%)	Result	Reference
In vitro					
Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2uvrA/ pKM101 (±S9), standard plate and preincubation test	5000 µg/plate −S9 5000 µg/plate +S9	PP/241024/2 + PP/241067/1; 97.8	Positive Positive	Stammberger (2000)
Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2uvrA/ pKM101 (±S9), standard plate and preincubation test	5000 µg/plate	OP2050190; 97.8	Negative	Ballantyne (2001a)
Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2uvrA/ pKM101 (±S9), standard plate and preincubation test	5000 µg/plate	OP2050045; 95.6	Negative	Ballantyne (2001b)
Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2uvrA/ pK M101 (±S9), standard plate and preincubation test	5000 µg/plate	OP2050046; 95.9	Negative	Ballantyne (2001c)
Gene mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2uvrA/ pKM101 (±S9), standard plate and preincubation test	5000 µg/plate	R001737; 99.3	Negative	Ballantyne (2001d)
Gene mutation	Chinese hamster lung V79 cells *hprt* locus	50 µg/ml −S9 30 µg/ml +S9	PP/241024/2 + PP/241067/1; 97.8	Negative Negative	Gräser & Stammberger (2000)

Table 23 (contd)

End-point	Test object	Concentration/dose (LED/HID)	Batch; purity (%)	Result	Reference
Chromosomal aberration	Chinese hamster lung V79 cells	3.2 µg/ml −S9 100 µg/ml +S9	PP/241024/2 + PP/241067/1; 97.8	Positive Positive	Stammberger & Gräser (2000)
Chromosomal aberration	Human peripheral blood lymphocytes	156 µg/ml −S9 625 µg/ml +S9	OP2050046; 95.9	Negative Negative	Allais (2001)
In vivo					
Unscheduled DNA synthesis	Male Sprague-Dawley rat, hepatocytes	2000 mg/kg bw × 1, 2 + 14 h after dosing	PP/241024/2 + PP/241067/1; 97.8	Negative	Mason (2000)
Micronucleus formation	Male and female NMRI mice, bone marrow cells	2000 mg/kg bw × 2 i.p., 24 h after dosing	PP/241024/2 + PP/241067/1; 97.8	Negative	Roth (2000)
Micronucleus formation	Male and female CD-1 mice, bone marrow cells	2000 mg/kg bw × 2 p.o., 24 h after second dose	OP2050046; 96.1	Equivo-cal	Whitwell (2003)
Micronucleus formation	Male and female NMRI mice, bone marrow cells	600 mg/kg bw × 2 i.p., 24 h after dosing	OP235005; 99.4	Negative	Herbold (2003a)

DNA, deoxyribonucleic acid; HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; p.o., per os (by mouth); S9, 9000 × *g* rat liver supernatant

A chromosomal aberration assay was also performed in Chinese hamster V79 cells with a mixture of batches PP/241024/2 and PP/241067/1. Significant increases in chromosomal aberrations (including and excluding gaps) were observed in two experiments. The first of these was performed in the presence and absence of S9, and the significant responses were observed at a concentration of 100 µg/ml and exposure time of 3 h in both activation conditions. The confirmatory experiment was performed only in the absence of S9, where the minimum significant effect concentration was 3.2 µg/ml and exposure was for 20 h. The treatment conditions were within the acceptable limits for the assay in the performing laboratory; thus, the mitotic indices at the highest concentrations were 38% and 48% in the absence and presence of S9, respectively, at 100 µg/ml in the first experiment and 22% at 6.3 µg/ml (64% at 3.2 µg/ml) in the second experiment. These indices indicate cytotoxicity, but it was not excessive.

A second chromosomal aberration assay was performed, but this time in human lymphocytes, and, unfortunately, the same batch of fluopicolide was not used. The human lymphocyte assay tends to be less sensitive than the assay with cell lines. In the first experiment with 3 h exposures, there was a small, dose-related increase in the percentage of aberrant cells in the presence of S9 that was in excess of the historical control range (upper 99% confidence limit) at all test dose levels against a relatively high background control value. This finding was not replicated in the second experiment in which exposures were for 3 h in the presence of S9 and 21 h in the absence of S9. The findings of the chromosomal aberration study in human lymphocytes were considered by the performing laboratory to show no evidence of clastogenicity. As the results of the first experiment were not reproduced, it is concluded that they represent an unconfirmed observation and are therefore considered of equivocal significance at most.

An in vivo rat hepatocyte unscheduled deoxyribonucleic acid (DNA) synthesis assay gave no evidence for genotoxicity following expression periods of 2 h and 14 h and single oral dose levels of up to 2000 mg/kg bw. Three studies of micronucleus induction were performed in bone marrow cells of male and female mice. There was no evidence of a significant response in two of these studies,

Table 24. Individual CD-1 mouse data from a bone marrow micronucleus test with fluopicolide

Dose (mg/kg bw per day × 2)	Total polychromatic erythrocyte count	No. of micronucleated polychromatic erythrocytes	No. of micronucleated polychromatic erythrocytes/1000 polychromatic erythrocytes
0	4000	3	0.75
	4000	9	2.25
	4000	1	0.25
	4000	2	0.52
	4000	3	0.75
	4000	3	0.75
2000	4000	2	0.50
	4000	5	1.25
	4000	11	2.75
	4000	3	0.75
	4000	7	1.75
	4000	8	2.00

From Whitwell (2003)

including one in which the batch mixture PP/241024/2 + PP/241067/1 that had been associated with mutation in bacteria and clastogenesis in V79 cells was tested up to the limit dose of 2000 mg/kg bw (Roth, 2000). In the third study, with batch No. OP2050046, there was a slightly elevated proportion of micronucleated cells at the single exposure dose of 2000 mg/kg bw.

Two in vivo micronucleus assays were performed in mice in which exposure was by the oral route in one and the intraperitoneal route in the other, up to the limit dose of 2000 mg/kg bw. In the first study (Roth, 2000), at three intraperitoneal dose levels of 2000 mg/kg bw per day and above, there was clearly no evidence for the proportion of micronucleated cells increasing with treatment, whereas the second study (Whitwell, 2003) at a single oral limit dose of 2000 mg/kg bw per day showed a higher proportion of micronucleated polychromatic erythrocytes in bone marrow of male mice than in the vehicle control group (0.88 ± 0.70 standard deviation [SD] per 1000 cells versus 1.50 ± 0.84 SD per 1000 cells). There was no evidence for bone marrow toxicity. The difference in the proportion of micronucleated cells was not statistically significant, although the difference was close to 2-fold. It was noted that the control value was particularly high in this experiment, the mean (of 11 experiments) historical value being 0.40 per 1000 cells. This deviation from the expected value presents difficulties in evaluating the effect of treatment. In an attempt to facilitate this process, the individual mouse values are presented in Table 24. The results highlight the weakness in the approved limit dose design (single sex, single dose), where the objective is to reduce the numbers of animals used in an experiment, but at the cost of losing any possibility of observing intergroup variations in response.

Most of the high vehicle control value (0.88 per 1000) is due to a single mouse. Without this mouse, the mean value would have been 0.60 per 1000. In the 2000 mg/kg bw group, there were four values (out of six) that might be considered as high. Considering the unclear biological and statistical significance of the findings, this study is considered to be equivocal.

A third micronucleus assay was performed in an attempt to determine the genotoxic potential of fluopicolide in vivo (Herbold, 2003a). There was clearly no significant response in this assay, although it differed from the Whitwell (2003) study in several respects: the mouse strain was NMRI in place of CD-1; the batch No. was OP235005 (purity 99.4%) in place of OP2050046 (purity 96.1%); and the high dose used was 600 mg/kg bw delivered by the intraperitoneal route in place of 2000 mg/kg bw delivered orally. Although these differences cause difficulties in interpretation, it is noted that the

Table 25. Doses received by rats in different segments of the two-generation study of fluopicolide

	Doses[a] (mg/kg bw per day)					
	F_0 generation			F_1 generation		
	Dietary concentration (ppm)					
	100	500	2000	100	500	2000
Males, pre-pairing	5.2	25.5	103.4	5.7	28.3	117.1
Females, pre-pairing	6.4	32.9	127.3	6.8	34.6	141.6
Females, gestation	7.1	35.8	145.4	7.3	37.2	147.0
Females, lactation	10.1	50.6	214.6	12.8	58.6	252.2

From Blee (2003)

[a] All doses are the lowest recorded within the time periods mentioned.

intraperitoneal route causes more systemic toxicity than the oral route, and there was evidence for bone marrow toxicity that was statistically significant at 600 mg/kg bw.

In summary, the overall weight of evidence suggests that fluopicolide has weak clastogenic properties in vitro but very weak clastogenic potential in vivo that may be batch specific. The high-purity material is considered unlikely to present a genotoxic hazard to humans.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study of reproduction in rats, groups of male and female Crl:CD (SD) IGS BR strain rats were administered fluopicolide (purity 95.9%; batch No. OP2050046) continuously in the feed at dietary levels of 0, 100, 500 or 2000 ppm throughout the two generations. These dietary concentrations were equal to the doses given in Table 25.

The F_0 generation comprised 28 males and 28 females in each dose group. They received the treated diet for 10 weeks before pairing and throughout mating, gestation, littering and lactation. Offspring survival and growth to weaning were evaluated, at which point 24 male and 24 female offspring per dose group were selected to form the F_1 generation. Both sexes received similar treated diets as their parents for a minimum of 10 weeks from selection, throughout pairing, gestation, littering and lactation. Sexual maturation, fertility and reproductive capacity of the F_1 generation were assessed, and resulting F_2 offspring were monitored for survival and development until weaning.

All adult F_0 and F_1 rats were subjected to a detailed postmortem examination in which the reproductive organs and other selected organs were weighed and retained. Sperm motility and morphology were assessed from samples obtained from the left vas deferens, and sperm counts were determined for the left epididymis and testis for all F_0 and F_1 males in the 0 and 2000 ppm dose groups. Microscopic examinations were made on designated tissues from 10 parental males and 10 parental females in the 0 and 2000 ppm dose groups and on abnormal tissues from all other parental rats. F_1 and F_2 offspring not selected for further treatment were killed on day 34 of age. Where possible, one male and one female from each litter were subjected to necropsy, and the reproductive organs, brain, spleen and thymus were weighed and retained for histopathological investigation.

The mean concentrations of fluopicolide in the dietary formulations prepared for use during weeks l, 11 and 18 of treatment of the F_0 generation and weeks 8 and 17 of treatment of the F_1 generation ranged from 87.8% to 101% of nominal concentrations and were considered satisfactory.

The general condition of the F_0 males and females was satisfactory throughout the study, and no treatment-related clinical signs or treatment-related deaths were recorded. One F_0 female receiving 500 ppm was killed in extremis following parturition problems (dystocia). However, this death was considered to be unrelated to treatment.

Food consumption was generally slightly lower at 2000 ppm in males and females prior to pairing and in males throughout the treatment period, reflecting the reduced body weight gain observed during this period. In females, this pattern continued during the first 2 weeks of gestation. However, from day 13 of gestation through to day 6 of lactation, food consumption was similar to that of the controls. A further reduction in food consumption was observed between days 7 and 13 of lactation, possibly as a result of the lower offspring body weight observed during this period, resulting in less demand on the lactating dam. There was no evidence of an adverse effect of treatment with fluopicolide on food consumption at 500 or 100 ppm before pairing, during gestation or during lactation.

Mean body weight and cumulative body weight changes were lower in males at 2000 ppm throughout the treatment period, with differences frequently attaining statistical significance. In females, mean body weight and body weight changes were lower just prior to pairing, as well as during the first 13 days of the gestation period. Following parturition, there was no adverse effect of treatment on the pattern of body weight change. At dose levels of 100 and 500 ppm, no treatment-related effects on the mean body weights or cumulative body weight changes were observed.

Estrous cycle regularity was not affected at any dose level of fluopicolide. There was no evidence of an adverse effect of fluopicolide on mating performance or fertility, as assessed by precoital interval, percentage mating, conception rate and fertility index at any dose level. With the exception of one control female, two females at 100 ppm and one female at 500 ppm, all females were pregnant and gave birth to live offspring. There was no adverse effect of treatment with fluopicolide on gestation length or gestation index, the length of the gestation phase being between 22 and 23 days for females in all groups. There were no treatment-related difficulties during parturition.

There were no instances of total litter loss and, with the exception of the female at 500 ppm that was killed in extremis prior to day 1 of lactation, all females reared their litters to maturity. The number of uterine implantation sites, litter size at birth, survival of offspring to litter standardization on day 4 and subsequent survival to weaning did not provide any evidence of an effect of treatment. Assessment of the sex ratio from day 1 to day 21 after birth did not indicate any adverse effects of treatment with fluopicolide upon the survival of rats of either sex throughout this period.

Postmortem examination of F_0 parents did not reveal any notable abnormalities. Organ weights of F_0 parents revealed increased mean absolute and relative kidney and liver weights and reduced spleen weights in males and females at 2000 ppm; these instances often attained statistical significance, with the most notable and consistent difference being recorded for the liver (Table 26). There were no treatment-related changes in the absolute and relative organ weights in rats at dose levels of 500 ppm and below.

Sperm analysis and morphology of F_0 males by quantitative assessment of the sperm parameters (motility, progressive motility, sperm count, homogenization-resistant spermatids) using a computer-assisted sperm analyser and visual assessment of sperm morphology showed that these parameters were not affected by treatment with fluopicolide.

Microscopic examination of the organs and tissues taken from the F_0 males and females did not reveal any treatment-related effects. Qualitative examination of the primordial follicle populations of the F_0 females treated with fluopicolide showed that they were similar to those of the controls.

Vaginal smears taken post-weaning (days 25–28 of lactation) showed that most F_0 females had returned to normal estrous cycles and attained estrus before termination and that fluopicolide had no effect on estrous cycles.

Table 26. Absolute and relative to body weight organ weight changes for F_0 parents

Parameter	Male				Female			
	Dietary concentration (ppm)							
	0	100	500	2000	0	100	500	2000
Terminal body weight (g)	618.8	605.7	600.0	587.5	325.0	339.2	327.2	305.7*
Absolute liver weight (g)	22.49	23.44	22.54	26.94*	15.58	16.49	16.12	17.84**
Relative liver weight (%)	3.628	3.871*	3.756	4.594**	4.800	4.858	4.926	5.828**
Absolute kidney weight (g)	4.03	4.01	4.05	4.44*	2.65	2.81	2.63	2.67
Relative kidney weight (%)	0.654	0.663	0.678	0.757**	0.817	0.829	0.805	0.873**
Absolute spleen weight (g)	0.908	0.854	0.854	0.800**	0.613	0.634	0.591	0.524**
Relative spleen weight (%)	0.1473	0.1424	0.1429	0.1369	0.1878	0.1874	0.1804	0.1719*

From Blee (2003)
* $P < 0.05$; ** $P < 0.01$

Although initial group mean body weight values were similar in all dose groups, both male and female F_1 offspring of the 2000 ppm group developed a similar pattern of significantly reduced body weight gain from day 7 of age through to weaning, as was observed in the F_0 rats at this dosage level. There was no evidence of an adverse effect of treatment with fluopicolide on offspring body weight or body weight change from birth through to weaning at dose levels of 500 ppm and below.

The nature and incidence of findings observed upon postmortem examination of offspring dying before weaning and the unselected offspring killed at weaning did not suggest any adverse effect of treatment with fluopicolide. The majority of the offspring dying before weaning presented with no milk in the stomach. This is a common finding in such offspring and is considered to reflect a possible lack of maternal care. As there was no association of offspring deaths with treatment, the assumed lack of maternal care also would appear to be unassociated with treatment. The incidence of pups dying after litter standardization was negligible, and there was no evidence of an increase in mortality associated with transition to treated diet.

Organ weights of unselected F_1 offspring showed slightly, but significantly, lower absolute spleen and thymus weights in males and females at 2000 ppm, which were largely related to the lower terminal body weight observed at this dosage. Thus, the relative organ weight values for spleen and thymus in male offspring were similar to those of the controls, as were the relative thymus weights for female offspring; however, relative spleen weight values were slightly lower than those of controls (92% of the controls). There were no organ weight changes at dose levels of 100 and 500 ppm in offspring dying before weaning and the unselected offspring killed at weaning of the F_1 generation.

There were no treatment-related clinical signs or unscheduled deaths in the F_1 generation, and the general condition of the F_1 rats was satisfactory.

Food consumption in males at 2000 ppm was slightly lower than that of controls from week 1 to week 8, but had recovered by week 10 before pairing of the F_1 generation. In females, food consumption was lower during the pre-pairing period, and this pattern continued throughout the gestation period in particular, when consumption was 11–16% lower than in the controls, and the lactation periods, when consumption was 8–10% lower than in the controls. There was no evidence of an adverse effect of treatment with fluopicolide on food consumption in both sexes at dietary concentrations of 100 and 500 ppm.

In the post-weaning early life of the F_1 generation, body weights in males and females at 2000 ppm were lower than those of controls, reflecting the pattern established before weaning. Body weights of this group remained consistently lower than those of the controls throughout the

generation, frequently attaining statistical significance in the period up to approximately 8 weeks of age. In females, body weights of the 2000 ppm group continued to be low throughout the pre-pairing and gestation phases. However, following parturition, there was no evidence to suggest that treatment with fluopicolide affected the normal pattern of body weight change during the lactation period, with overall gain by weaning being superior to that of the concurrent control. There was no evidence of an adverse effect of treatment with fluopicolide on body weight or body weight change prior to pairing, during gestation or during lactation at dose levels of 100 and 500 ppm.

Sexual maturation was not affected by treatment with fluopicolide, and there were no significant intergroup differences in the time of completion of vaginal opening or balano-preputial separation at any dosage level. Estrous cycles, mating performance and fertility, as assessed by precoital interval, percentage mating, conception rate and fertility index, did not show any treatment-related intergroup differences. Gestation length, gestation index and parturition were not affected by treatment with fluopicolide. With the exception of one female at 2000 ppm, all females were pregnant and gave birth to live offspring. There was no adverse effect of treatment with fluopicolide on gestation length or gestation index, with the length of the gestation phase being between 22 and 23 days for females in all groups. There were no difficulties evident during the parturition process that were considered to be related to treatment.

One female of the 2000 ppm group failed to become pregnant. Two females experienced total litter loss, one at 500 ppm on day 4 of lactation and one at 2000 ppm on day 3 of lactation. It was noted that most coincidental instances of total litter loss in the CD rat occur during this period, and the isolated incidences in this study were probably not related to treatment with fluopicolide. The numbers of litters surviving to weaning were 24, 24, 23 and 22 at 0, 100, 500 and 2000 ppm, respectively. There were no treatment-related effects on the numbers of uterine implantation sites (recorded at termination), litter size at birth, survival of offspring to litter standardization on day 4 and subsequent survival to weaning. Sex ratios from day 1 to day 21 after birth did not indicate any adverse effects of treatment with fluopicolide upon the survival of rats of either sex throughout this period. Body weights of offspring at birth were similar in all dose groups. However, F_2 male and F_2 female offspring of the 2000 ppm group showed a similar pattern of lower body weight gain from the second week of age. Body weight gains of the F_2 offspring treated at dose levels of 100 and 500 ppm were not affected by treatment.

Postmortem examination of F_2 offspring at 34 days of age did not reveal any abnormalities. Measurement of organ weights of these rats revealed lower absolute spleen and thymus weights in males and females that were very similar to those observed in the F_1 generation. Relative spleen weight was also lower in females of the 2000 ppm dose group, but no treatment-related organ weight changes were observed at dose levels of 100 and 500 ppm.

Postmortem examination of F_1 parents did not reveal any treatment-related abnormalities. Increased absolute and relative liver and kidney weights were observed in both males and females at 2000 ppm. The females in this group also had 15% lower absolute spleen weights, but the relative spleen weights were not significantly different from controls. Relative liver weights in females, but not in males, were slightly (8%) but significantly increased at 500 ppm compared with controls.

Sperm analysis and morphology of F_1 males showed that the numbers of motile and progressively motile sperm (from the vas deferens) and the numbers of caudal epididymal sperm and testicular spermatids were similar in all groups, and no treatment-related effects upon spermatogenesis or sperm maturation were observed.

Microscopic examination of the organs and tissues taken from the F_1 males and females did not reveal any treatment-related findings. Vaginal smears of F_1 females taken post-weaning (days 25–28 of lactation) showed that most females had returned to estrous cycling and attained estrus before termination. Ovarian primordial follicle counts of F_1 females did not reveal any treatment-related intergroup differences.

In conclusion, dietary administration of fluopicolide at concentrations of 100, 500 or 2000 ppm was generally well tolerated by the F_0 and subsequent F_1 parental rats and their respective progeny. In the F_0 and F_1 generations, lower feed intake and a corresponding reduction in body weight gain were noted at 2000 ppm following the start of treatment and persisted throughout the generation. Fertility and reproductive performance of the F_0 and F_1 parental rats were unaffected by treatment, and litter parameters at birth of the F_1 and F_2 progeny and their survival to weaning showed no adverse effects of treatment. No abnormal findings were observed during postmortem examination of the F_0 or F_1 parental rats, the post-weaned unselected offspring or the F_2 offspring. Increased kidney and liver weights were observed in parental rats treated at 2000 ppm in both generations. Detailed examination of reproductive tissues and gross abnormalities did not reveal any treatment-related findings at 2000 ppm, and sperm analysis in both generations did not reveal any treatment-related findings.

The NOAEL for parental toxicity was 500 ppm, equal to 25.5 mg/kg bw per day for males and 32.9 mg/kg bw per day for females, based on liver and kidney weight increases at 2000 ppm, equal to 103.4 mg/kg bw per day for males and 127.3 mg/kg bw per day for females. The NOAEL for reproductive toxicity in the multigeneration study in rats was 2000 ppm, equal to 103.4 mg/kg bw per day for F_0 males and 127.3 mg/kg bw per day for F_0 females, for the period before pairing, the highest dose tested. The overall NOAEL for pups and developing offspring was 500 ppm, equal to 25.5 mg/kg bw per day for males and 32.9 mg/kg bw per day for females, based on reduced body weight gains of pups during lactation and reduced absolute spleen and thymus weights in males and females of the F_1 and F_2 generations at 2000 ppm, equal to 103.4 mg/kg bw per day for F_0 males and 127.3 mg/kg bw per day for F_0 females, for the period before pairing (Blee, 2003).

It is concluded that fluopicolide is not a selective reproductive toxin.

(b) Developmental toxicity

Rats

In a range-finding study, designed to select suitable doses of fluopicolide for a subsequent developmental toxicity study, groups of four mated female Sprague-Dawley rats were administered fluopicolide (purity 97.6%; batch mixture PP/241024/2 and PP241067/1) by gavage in aqueous methyl cellulose (1% w/v) at dose levels of 0, 500 or 1000 mg/kg bw per day from day 7 to day 20 of pregnancy (day 0: day of mating; day 1: day of sperm detection) and were killed on day 21 of pregnancy. Rats were observed daily for mortality and clinical signs of toxicity. Body weight and food consumption were determined regularly throughout the study. At postmortem examination, the dams were examined for gross changes, after which the uterus was opened and the number of live and dead fetuses and the number of conceptuses undergoing resorption were counted. Body weights, crown–rump lengths and placental weights were measured.

There were no deaths during the study. Food consumption showed a marked initial decrease in the 1000 mg/kg bw per day dose group and between days 7 and 10 at 500 mg/kg bw per day. Body weight gain was reduced in the 1000 mg/kg bw per day group throughout the treatment period. No gross changes were observed at necropsy. The incidence of postimplantation loss was increased at 1000 mg/kg bw per day. Total litter loss occurred in one rat from this group; in addition, another female had a high incidence of dead implantations. Mean fetal weight and crown–rump length values were reduced at 1000 mg/kg bw per day. Slight decreases in fetal weights and fetal crown–rump lengths were also observed at 500 mg/kg bw per day.

Based on the findings of this study, the appropriate highest test dose in the main study was considered to be between 500 and 1000 mg/kg bw per day (Hofmann, 2000a).

In an oral developmental toxicity study, groups of 23 mated female Sprague-Dawley rats were administered fluopicolide (purity 97.6% and 97.8% for the respective components of batch mixture

Table 27. Body weight changes of dams during pregnancy in a developmental toxicity study in rats

Time period	Body weight changes (g)			
	Dose level (mg/kg bw per day)			
	0	5	60	700
Days 1–4	8.4	7.1	7.4	4.8
Days 4–7	12.7	14.3	11.9	13.8
Days 7–10	10.8	11.0	11.2	8.2
Days 10–14	16.0	15.2	15.1	14.0
Days 14–17	26.0	25.2	23.6	23.8
Days 17–19	29.0	28.8	30.3	28.0
Days 19–21	32.9	37.2	34.0	31.2

Table 28. Body weights, crown–rump length and placental weights in a developmental toxicity study in rats

Parameter	Dose level (mg/kg bw per day)			
	0	5	60	700
Fetal weight (g)	3.7	3.7	3.6	3.4*
Crown–rump length (mm)	36.2	36.0	36.1	34.8*
Placental weight (g)	0.57	0.53	0.53	0.52*

From Hofmann (2001a)
* $P < 0.05$

PP/241024/2 and PP241067/1) by gavage in 1% (w/v) methyl cellulose once daily at dose levels of 0, 5, 60 or 700 mg/kg bw per day from day 7 to day 20 of pregnancy (day 0: day of mating; day 1: day of sperm detection) and were killed on day 21 of pregnancy. Behaviour and state of health of the rats were observed daily. Body weight and food consumption were determined regularly throughout the study. At necropsy, the dams were examined for gross changes. The weights of the unopened gravid uteri were recorded. The uteri were opened, and the numbers of live and early or late dead fetuses were recorded. Body weights, crown–rump lengths, sex ratios of the fetuses and placental weights were measured. The fetuses were examined for external, visceral and skeletal anomalies.

There were no deaths during the study, and no clinical signs were observed in any of the rats. Body weight gains were slightly but not statistically significantly decreased at 700 mg/kg bw per day at the beginning of gestation and after the beginning of substance administration (Table 27), whereas food consumption was not significantly decreased.

Necropsy of the dams did not reveal any treatment-related abnormalities. Gravid uterine weights and incidences of dead early and late conceptuses were comparable in all dose groups.

Mean fetal body weights, crown–rump lengths and placental weights were slightly decreased in the 700 mg/kg bw per day dose group (Table 28). Litter size, number of live and dead fetuses as well as sex ratios were unaffected by treatment.

The external, skeletal and visceral examination of fetuses revealed no treatment-related major defects. There was one fetus with multiple malformations of the vertebral column and the pelvis in

the 60 mg/kg bw per day dose group and one fetus with microphthalmia in the 700 mg/kg bw per day dose group. These sporadic and low-incidence findings are considered to be unrelated to treatment. Minor skeletal anomalies observed more frequently in the 700 mg/kg bw per day dose group consisted of aplastic, dysplastic or fused thoracic vertebral arches (0/148, 0/150, 1/153, 4/142), aplastic, dysplastic, fragmented, fused or dislocated thoracic vertebral centres (0/148, 0/150, 1/153, 10/142*), fragmented or longitudinally displaced sternebrae (0/148, 0/150, 0/153, 3/142), aplastic, dysplastic, shortened or fused ribs or consisting of only nine ribs (0/148, 0/150, 1/153, 6/142) as well as wavy and/or thickened ribs (1/148, 1/150, 0/153, 5/142). These incidences were statistically significant (* $P < 0.05$) or distinctly higher than the upper limit of the tolerance interval of the historical range and therefore considered to be treatment related. In all other cases, statistical evaluation did not reveal differences between the groups, and the incidences were within or only slightly above the historical tolerance limit. Therefore, a treatment-related effect was not evident. Examination of body cross-sections did not reveal statistically significant changes; the incidences of any changes were within or only slightly above the historical tolerance limit.

One notable variation observed was the incidence of an extra rib at the seventh cervical vertebra, which was above the upper limit of the historical confidence interval in the high dose group. However, the difference between the control and high dose groups was low, and statistical evaluation did not reveal differences between the groups. Therefore, a compound-related effect is questionable. In all other cases, the incidences were within or slightly above the historical tolerance interval of the historical range.

Retardations were observed in 700 mg/kg bw per day group fetuses and included increased incidences of ossification of less than two caudal vertebral centres, weakly or non-ossified sternebrae and non-ossified metacarpal 5, metatarsal 5 and phalanx III of the first to fifth toes. There were no treatment-related retardations at dose levels of 60 mg/kg bw per day and below.

The NOAEL for maternal toxicity and fetotoxicity was 60 mg/kg bw per day, based on slightly decreased body weight in dams and reduction in mean fetal body weights and crown–rump lengths in fetuses at 700 mg/kg bw per day. Further evidence of fetotoxicity at this dose was increased incidences of minor defects of the thoracic vertebrae, sternebrae and ribs, as well as delayed ossification. Fluopicolide was not teratogenic in the developmental toxicity study in rats (Hofmann, 2001a).

Rabbits

In a dose range–finding study to select suitable doses of fluopicolide for a subsequent developmental toxicity study, groups of four mated female Himalayan rabbits were administered fluopicolide (purity 97.6%; batch mixture PP/241024/2 and PP241067/1) by gavage in aqueous methyl cellulose (1% w/v) at dose levels of 0, 25, 50, 100, 250, 500 or 1000 mg/kg bw per day from day 6 to day 28 of pregnancy (day 0: day of mating) and were killed on day 29 of pregnancy. Rabbits were observed daily for mortality and clinical signs of toxicity. Body weight and food consumption were determined regularly throughout the study. At postmortem examination, the dams were examined for gross changes, after which the uterus was opened and the number of live and dead fetuses and the number of conceptuses undergoing resorption were counted. Body weights, crown–rump lengths and placental weights were measured.

All rabbits from the 100, 250, 500 and 1000 mg/kg bw per day dose groups were found dead, killed while in a moribund condition or killed after abortion up to day 23 of the study. These rabbits showed nonspecific symptoms, including impairment of motility and consciousness, respiratory sounds, decreased defecation and hay consumption, hyperactivity, hypoactivity and discoloured urine. At the dose of 50 mg/kg bw per day, one rabbit showed decreased defecation and discoloured tray on day 27. This rabbit aborted on day 28. The other rabbits in the 50 mg/kg bw per day dose group did not show any clinical signs of toxicity. There were no clinical signs of toxicity at 25 mg/kg bw per day. Reduced food consumption and body weight gain were observed at dose levels of

100, 250, 500 or 1000 mg/kg bw per day. Body weight gain was not impaired in rabbits at dose levels of 25 and 50 mg/kg bw per day. Food consumption was decreased at the end of the study in rabbits receiving 50 mg/kg bw per day.

Findings in the rabbits found dead, killed moribund or killed after abortion consisted of beige discoloured heart, liver and kidney. In most rabbits, the stomach showed petechial bleeding, and in some, it was filled with feed mash. No grossly visible changes were observed in rabbits of the 25 and 50 mg/kg bw per day groups. One rabbit at 25 mg/kg bw per day and one at 50 mg/kg bw per day were not pregnant. The rabbit at 50 mg/kg bw per day that aborted had six dead fetuses. No abnormalities were observed at caesarean section of the remaining rabbits from these groups. Gravid uterus and fetal weights were normal, and embryo/fetal development was unaffected. Based on the results of this study, a maximum dose level of around 50 mg/kg bw per day was considered appropriate for the main study (Hofmann, 2000b).

In a developmental toxicity study, groups of 23 mated female Himalayan rabbits were administered fluopicolide (purity 97.6%; batch mixture PP/241024/2 and PP241067/1) by gavage in aqueous methyl cellulose (1% w/v) at dose levels of 0, 5, 20 or 60 mg/kg bw per day from day 6 to day 28 of gestation (day 0: day of mating) and were killed on day 29 of gestation. Rabbits were observed daily for mortality and clinical signs of toxicity. Body weight and food consumption were determined regularly throughout the study. At postmortem examination, the dams were examined for gross changes, after which the uterus was opened and the number of live and dead fetuses and the number of conceptuses undergoing resorption were counted. Body weights, crown–rump lengths and placental weights were measured. The fetuses were examined for external, visceral and skeletal anomalies.

Three rabbits in the 60 mg/kg bw per day dose group were found dead (one on each of days 24, 25 and 29), and 15 rabbits of this group were killed after premature delivery during days 22–29 of gestation. These rabbits showed decreased defecation, reduced hay consumption, hypoactivity, bristling coat, pultaceous faeces and discoloured urine within 1–4 days before premature delivery. One rabbit of this dose group showed increased salivation. One rabbit in the 20 mg/kg bw per day group was killed after premature delivery on day 28 of gestation; it had shown decreased defecation and reduced hay consumption.

Food consumption and body weight gain were markedly reduced in the 60 mg/kg bw per day dose group rabbits throughout the treatment period, the reduction in food consumption reaching statistical significance on days 26 and 29 of gestation. Body weight and food consumption were comparable with those of controls at dose levels of 5 and 20 mg/kg bw per day.

Gravid uterine weights were 19% lower in the 60 mg/kg bw per day group. Gross postmortem examination of dams of this group showed overfilled stomach, red liquid in the urinary bladder and uterus and yellowish discoloration of the liver.

In most cases in which premature delivery occurred, dead fetuses were observed. With the exception of three females at 5 mg/kg bw per day and one female at 20 mg/kg bw per day, all rabbits became pregnant. There were no treatment-related changes in the incidence of early and late deaths of conceptuses.

Mean fetal body weights and crown–rump lengths were statistically significantly decreased by 14% and 6%, respectively, in fetuses at 60 mg/kg bw per day. Litter size, number of live fetuses and the sex ratio were not affected by treatment. External, skeletal and visceral examination of the fetuses did not reveal any treatment-related abnormalities.

The NOAEL for fetotoxicity and maternal toxicity in rabbits was 20 mg/kg bw per day, based on mortality, high incidence of premature delivery and reduction in body weight gain and food consumption in dams and reduction in fetal body weights and fetal crown–rump lengths at a dose of 60 mg/kg bw per day. Fluopicolide was not teratogenic in the developmental toxicity study in rabbits (Hofmann, 2001b).

2.6 Special studies

(a) Neurotoxicity

In a single-dose neurotoxicity study, groups of 10 male and 10 female overnight-fasted CD rats were administered fluopicolide (purity 95.9%; batch No. OP2050046) by gavage in 1% aqueous methyl cellulose at dose levels of 0, 10, 100 or 2000 mg/kg bw. Dose selection was based on a preliminary range-finding study for benchmark dose and time to peak effect determination. Neurobehavioural screening, consisting of an FOB of tests and motor activity examinations, was performed before commencement of dosing and on days 1 (6 h after dosing), 8 and 15. Body weights were recorded pretreatment, immediately before dosing, on days 8 and 15 and before necropsy. A body weight recording was also made each time an FOB was performed. Food consumption was recorded weekly. On completion of the study, the rats were killed and subjected to gross examination; the brain was measured for length and width and weighed. Selected tissues, including the brain and nervous tissues, were retained from all rats and examined from five males and five females in the 0 and 2000 mg/kg bw dose groups. The liver, the principal target organ, was not collected or examined.

There were no deaths during the study. No signs of toxicity were seen during routine observations, and body weights, food consumption, food conversion efficiency and brain weights were unaffected by treatment.

Gross and microscopic examination of the organs and tissues did not reveal any treatment-related effects. Low body temperatures were observed in rats at 2000 mg/kg bw, and there was a higher incidence of excessive grooming in females at this dose level.

The NOAEL in the neurotoxicity screening study in rats was 100 mg/kg bw, based on reduction in body temperature and increased incidence of excessive grooming in females at 2000 mg/kg bw (Cooper, 2002a).

In a 2002 GLP-compliant subchronic neurotoxicity study, three groups of 10 male and 10 female CD rats received fluopicolide (purity 97.8%; batch No. OP2050046) orally, via the diet, at concentrations of 0, 200, 1400 or 10 000 ppm, equal to 0, 15.0, 106.6 and 780.6 mg/kg bw per day for males and 0, 18.0, 125.2 and 865.8 mg/kg bw per day for females, for 13 weeks. Rats were inspected at least twice daily for evidence of reaction to treatment or ill-health. A more detailed weekly examination was also performed on each rat. Body weights were recorded before study start, the first day of treatment, weekly throughout the treatment period and before necropsy. Food consumption was recorded weekly throughout the study. Neurobehavioural screening, consisting of an FOB of tests, was performed before study start and during weeks 4, 8 and 13 on all rats. At termination, rats were killed and subjected to a detailed necropsy. The brain was dissected, weighed and fixed for microscopic examination. Kidneys, adrenals, liver and bone sections were also preserved for microscopic examination. For microscopy, five males and five females from the control and high dose groups were selected.

There were no deaths during the study. The appearance and behaviour of the rats were normal and not affected by treatment. Overall body weight gains were reduced in females at dose levels of 1400 ppm (13%) and 10 000 ppm (28%) and in males at 10 000 ppm (19%) compared with controls. Overall food consumption was slightly lower for males (7%) and females (8%) receiving the 10 000 ppm diet.

Neurobehavioural screening did not reveal any treatment-related changes. Home cage observations, in-the-hand observation, arena observations and manipulations did not reveal any treatment-related changes. The group mean motor activity scores showed no consistent dose- or treatment-related changes, and none of the differences achieved statistical significance. Brain weights and anatomical measurements of the cerebral hemispheres did not indicate any differences between the controls and

treated rats. Gross examination of rats killed on completion of the treatment period revealed no treatment-related findings. There were no changes in the tissues presented for neuropathological examination that were considered to be related to treatment with fluopicolide.

Treatment-related effects were seen in the liver and kidneys that were similar to those observed in other multiple-dose studies with fluopicolide. An increased incidence of centrilobular hepatocyte hypertrophy was observed in males (from 0/10 in the controls to 9/10 at 1400 ppm and 10/10 at 10 000 ppm) and in females (from 0/10 in the controls to 6/10 at 10 000 ppm). In the kidneys of male rats, there was an increase in the incidence and/or severity of hyaline droplets in the cortical tubules in the kidneys of all males at dose levels of 1400 ppm and 10 000 ppm. Also at 10 000 ppm in the kidneys of male rats, there were high incidences of interstitial inflammation (9/10), granular casts (10/10) and cortical tubule dilatation (7/10). In female rats, there were no incidences of these features, except for interstitial inflammation in 1 of 10 rats of the 1400 ppm group.

The NOAEL for systemic toxicity in the 13-week neurotoxicity study was 200 ppm, equal to 15.0 mg/kg bw per day in males and 18.0 mg/kg bw per day in females, based on impaired growth and histopathological changes in the liver and kidney at 1400 ppm, equal to 106.6 mg/kg bw per day in males and 125.2 mg/kg bw per day in females. The NOAEL for neurotoxicity was 10 000 ppm, equal to 781 mg/kg bw per day in males and 866 mg/kg bw per day in females, the highest dose tested (Cooper, 2002b).

(b) Mechanistic studies on the oncogenic action in mice

In a study designed to investigate short-term liver changes in mice at the dose of fluopicolide associated with an increased incidence of hepatocellular adenomas, particularly in females after 78 weeks of exposure, and to allow comparison with separate studies (see below) in which mice were treated with phenobarbital or clofibrate, groups of 35 female C57BL/6 mice were administered fluopicolide (99.3% purity; batch No. R001737) in the diet at concentrations of 0 or 3200 ppm, equal to 0 or 575 mg/kg bw per day, for either 7 days (20 mice per goup) or 28 days (15 mice per group). Clinical signs were recorded daily, and body weight and food consumption were measured weekly. A detailed physical examination was performed weekly. Bromodeoxyuridine (BrdU) was administered in drinking-water for 7 days before the mice were scheduled to be killed for cell proliferation assessment. Water consumption was measured during the period of BrdU administration. At both the 7-day and 28-day study termination times, the livers were weighed and sampled. Hepatic cellular proliferation and histology were assessed. In addition, hepatic cytochrome P450 isoenzyme activities were assessed at 7 days.

There were no mortalities or clinical signs of toxicity during the study in the 3200 ppm dose group. Mean food consumption in this group was lower than in the controls between days 1 and 7 (−25%), but not over days 7–28. A body weight loss of about 10% was observed over days 1–7, but body weights were stable in both the 0 and 3200 ppm groups for the remainder of the study.

For those mice killed on day 7, mean terminal body weight was statistically significantly lower in the 3200 ppm group by about 7%. Mean absolute and relative liver weights were increased by 27% and 37% in this same group. Gross examination revealed livers that were dark (9/20) and enlarged (1/20). Histopathological examination of the liver showed diffuse, perilobular to panlobular hepatocellular hypertrophy in 20 of 20 treated mice, compared with 0 of 20 controls, and a marked loss of diffuse, mainly centrilobular hepatocellular vacuolation, which was observed in 3 of 20 treated mice, compared with 20 of 20 controls. An increased number of mitotic cells and some foci of single-cell necrosis/apoptosis were seen in 5 of 20 treated mice.

For those mice killed on day 28, mean terminal body weight was not affected. Mean absolute and relative liver weights were increased by 48% and 56% compared with controls. Macroscopic examination at necropsy revealed livers that were dark (11/15) and enlarged (3/15). There was diffuse, perilobular to panlobular hepatocellular hypertrophy in 15 of 15 treated mice, compared with

Table 29. Mean BrdU labelling index at 7 and 28 days in female mice treated with fluopicolide, phenobarbital or clofibrate

	Mean BrdU labelling index (no. of BrdU-positive cells out of 1000 cells)				
	Fluopicolide		Control	Phenobarbital	Clofibrate
	Dietary concentration (ppm)		Dose level (mg/kg bw per day)		
	0	3200	0	80	300
Day 7 centrilobular	—	—	12.2	140.7**	136.8**
Day 7 perilobular	—	—	20.7	137.1**	147.8**
Day 7 total	23.55	152.95**	16.4	138.7**	142.5**
Day 28 centrilobular	—	—	29.8	25.8	16.4
Day 28 perilobular	—	—	29.1	31.2	42.5*
Day 28 total	29.62	17.00	29.4	28.7	30.0

From Langrand-Lerche (2004b)
* $P < 0.05$; ** $P < 0.01$ (Mann-Whitney exact test)

Table 30. Total cytochrome P450 content and enzymatic activities at 7 days in female mice after treatment with fluopicolide, phenobarbital or clofibrate

Parameter	Fluopicolide		Control	Phenobarbital	Clofibrate
	Dietary concentration (ppm)		Dose level (mg/kg bw per day)		
	0	3200	0	80	300
Total P450 (nmol/mg protein)	1.11	2.19**	1.25	2.21**	1.55
BROD (pmol/min per milligram protein)	57.3	1079.8*	63.1	1005.8*	41.8*
EROD (pmol/min per milligram protein)	71.2	127.2*	87.7	128.1	62.0
PROD (pmol/min per milligram protein)	18.3	227.4*	16.7	153.5*	9.4*
Lauric acid hydroxylase (nmol/min per milligram protein)	15.37	5.09*	7.40	5.81	15.68*

From Langrand-Lerche (2004b)
* $P < 0.05$; ** $P < 0.01$ (Mann-Whitney exact test)

0 of 15 controls, together with a marked loss of diffuse, mainly centrilobular hepatocellular vacuolation, which was observed in 3 of 15 treated mice, compared with 15 of 15 controls. Foci of single-cell necrosis or apoptosis were seen in 1 of 15 treated mice, and an increased number of mitotic cells was seen in 2 of 15 treated mice.

On day 7, the mean BrdU labelling index was approximately 6.5-fold higher in treated mice than in controls, which is indicative of marked hepatocellular proliferation (Table 29). In contrast, there was no hepatocellular proliferation at day 28.

In the liver, fluopicolide induced a marked increase in total cytochrome P450 content (+97%) and in benzoxyresorufin *O*-debenzylase (BROD) activity (+1785%) and pentoxyresorufin *O*-depentylase (PROD) activity (+1143%); there was also a smaller increase in ethoxyresorufin *O*-deethylase (EROD) activity (+79%). Lauric acid hydroxylation (a marker for peroxisomal proliferation) was reduced (Table 30).

This study demonstrated that dietary administration of fluopicolide at 3200 ppm induced increased liver weights, hepatocellular hypertrophy as well as a transient and marked hepatocellular proliferation in C57BL/6 mice after 7 days of treatment, which returned to control levels after 28 days of treatment. Fluopicolide also induced cytochrome P450 and BROD and PROD enzyme activities (Langrand-Lerche, 2004a).

In a study that permitted comparison with the one described above, groups of 35 female C57BL/6 mice were administered phenobarbital (sodium salt) (batch No. 088H0023) by gavage at 80 mg/kg bw per day, clofibric acid (batch No. 01220BT-12) at 300 mg/kg bw per day or the vehicle control (0.5% aqueous methyl cellulose) for either 7 days (15 mice per group) or 28 days (20 mice per group). The investigations conducted were as described for the experiment with fluopicolide (above).

Homogeneity, concentration and stability of phenobarbital and clofibric acid under study conditions were investigated. At the start of treatment, the mice were approximately 10 weeks old.

In the 80 mg/kg bw per day phenobarbital part of the study, no mortality occurred in phenobarbital-treated mice during the study. Reduced motor activity was observed during most of the treatment period in all mice immediately after the administration of the test substance by gavage. Mean body weight and body weight gain in females were not affected by the treatment.

In the groups of female mice killed at 7 and 28 days, the mean absolute and relative liver weights were increased compared with controls. Dark liver was observed in 10 of 20 females at 7 days and in 9 of 15 females at 28 days.

Microscopic examination showed a diffuse, centrilobular to midzonal hepatocellular hypertrophy in 18 of 20 females at 7 days and in all mice at 28 days. Additionally, at both times, a loss of centrilobular to diffuse hepatocellular vacuolation was seen in some female mice. After 7 days of treatment, a marked hepatocellular proliferation was noted and was considered to be treatment related: centrilobular, perilobular and global proliferation indices were 6.6- to 14.6-fold higher in treated mice than in the controls (Table 29). At 28 days, there were no significant changes in BrdU labelling index. At 7 days, phenobarbital markedly induced total cytochrome P450 and BROD and PROD activities, but there were no significant effects on EROD or lauric acid hydroxylase activities (Table 30).

In the 300 mg/kg bw per day clofibric part of the study, one male mouse was found dead on day 6. In addition, two males and two females were killed for humane reasons during the first 12 days of treatment. Of these, one male and one female showed clinical signs indicative of gavage errors that were confirmed at autopsy. Clinical signs observed for the other decedent mice were reduced motor activity, laboured respiration, soiled anogenital region, prostration, piloerection and coldness to touch. Mean body weight, body weight gain and food consumption were unaffected by treatment. Mean water consumption was increased by treatment between days 1–7 and days 22–28.

At both 7 and 28 days, mean absolute and relative liver weights were statistically significantly higher in female mice receiving clofibrate at 300 mg/kg bw per day. Dark liver was observed in 8 of 19 mice at 7 days and in 12 of 14 females at 28 days. A diffuse, centrilobular to panlobular hepatocellular hypertrophy was observed in all mice at both observation times. An increased number of mitotic hepatocytes was noted in 9 of 19 females at 7 days only. A loss of centrilobular to diffuse hepatocellular vacuolation was seen in the majority of mice at both observation times, which was associated with the persistence of a residual, diffuse periportal hepatocellular vacuolation in some mice. After 7 days of treatment, a marked hepatocellular proliferation was noted and was considered to be treatment related. Centrilobular, perilobular and global proliferation indices were 7.1- to 11.2-fold higher in treated mice, when compared with controls (Table 29). At 28 days, the perilobular proliferation index was statistically significantly higher and the centrilobular proliferation index was (non-significantly) lower, but the global proliferation index remained unchanged. At 7 days, clofibric acid significantly induced lauric acid hydroxylation, whereas BROD activity was significantly reduced (Table 30).

The experiment demonstrated that the oral administration of phenobarbital at 80 mg/kg bw per day induced a marked hepatocellular proliferation in female C57B1J6 mice after 7 days of treatment, and this effect returned to control levels after 28 days of treatment. In addition, phenobarbital strongly induced hepatocellular hypertrophy, total cytochrome P450 content and the activities of

Table 31. Total cytochrome P450 content and enzymatic activities at 7 days in male and female rats after treatment with fluopicolide or phenobarbital

Parameter	Fluopicolide		Phenobarbital	
	Dietary concentration (ppm)		Dose level (mg/kg bw per day)	
	0	2500	0	80
Males				
Total P450 (nmol/mg protein)	1.7	2.3*	1.8	4.1*
BROD (pmol/min per milligram protein)	13.2	467.6	36.6	943.5**
EROD (pmol/min per milligram protein)	62.6	108.4	48.4	95.9**
PROD (pmol/min per milligram protein)	13.4	118.1**	15.7	194.8**
Lauric acid hydroxylase (nmol/min per milligram protein)	2.5	1.4*	3.5	2.2
Females				
Total P450 (nmol/mg protein)	1.5	1.6	1.5	2.7*
BROD (pmol/min per milligram protein)	4.6	29.6	12.6	431.5**
EROD (pmol/min per milligram protein)	52.2	140.5**	58.9	60.1
PROD (pmol/min per milligram protein)	5.0	11.3**	5.5	69.1**
Lauric acid hydroxylase (nmol/min per milligram protein)	2.4	1.9	2.9	2.3

From Totis (2008)
* $P < 0.05$; ** $P < 0.01$

BROD and PROD. Clofibric acid at 300 mg/kg bw per day also induced a marked hepatocellular proliferation in female C57B1J6 mice after 7 days of treatment, which returned to control levels after 28 days of treatment. Although clofibric acid strongly induced hepatocellular hypertrophy and lauric acid hydroxylase activities, it had no significant effect on the total cytochrome P450 content of liver or on the hepatic activity of EROD and was associated with reduced activities of BROD and PROD. There seemed to be little similarity between the hepatic effects of fluopicolide and clofibrate, whereas there was a relatively close similarity between the effects of fluopicolide and phenobarbital (Langrand-Lerche, 2004b).

Hepatic enzyme experiments similar to those described for female mice were also conducted with male and female rats. The male and female Sprague-Dawley Crl:CD(SD) rats were administered diets containing 0 or 2500 ppm fluopicolide for 7 days, and two other groups received phenobarbital by gavage for 7 days at 0 or 80 mg/kg bw per day. The results of cytochrome P450 enzyme and uridine diphosphate (UDP)-glucuronosyltransferase activities are presented in Tables 31 and 32.

No treatment-related clinical signs were reported in rats treated with fluopicolide or phenobarbital. Body weights and food consumption were unaffected by treatment with the test compound or the positive control. No significant changes were noted in terminal body weights. Absolute and relative (to body weight) liver weights were slightly but significantly increased in both males and females treated with fluopicolide or phenobarbital.

Total cytochrome P450 was significantly increased only in male rats treated with fluopicolide, whereas there were increases in both male and female rats treated with phenobarbital. Considering only male rats, the increase in total cytochrome P450 was greater in rats treated with phenobarbital than in rats treated with fluopicolide. Activities of the associated enzymes PROD, BROD and EROD were markedly or significantly increased in male and female rats treated with fluopicolide. Although the activity profile for fluopicolide was similar to that for phenobarbital in male rats,

Table 32. UDP-glucuronosyltransferase activities at 7 days in male and female rats treated with fluopicolide or phenobarbital

Sex	UDP-glucuronosyltransferase activity (nmol/min per milligram protein)			
	Fluopicolide		Phenobarbital	
	Dietary concentration (ppm)		Dose level (mg/kg bw per day)	
	0	2500	0	80
Male	14.35	29.80**	16.89	23.93**
Female	7.76	15.47**	6.99	14.74**

From Totis (2008)
** $P < 0.01$

Table 33. Comparison of effects of fluopicolide and phenobarbital on enzymes in mice and rats

Enzyme[a]	Fluopicolide			Phenobarbital		
	Mouse F	Rat M	Rat F	Mouse F	Rat M	Rat F
CYP	2.0	1.4	1.1	1.8	2.3	1.8
BROD	19	35	6.4	16	26	34
EROD	1.8	1.7	2.7	1.5	2.0	1.0
PROD	12	8.8	2.3	9.2	12	13
LAH	0.3	0.6	0.8	0.8	0.6	0.8

CYP, cytochrome P450; LAH, lauric acid hydroxylase
[a] Enzyme gene families are as follows:
BROD CYP3A1/3A2
EROD CYP1A1/1A2
PROD CYP2B1/2B2 and CYP2E
LAH CYP4A

there were differences in female rats, in which EROD was increased following fluopicolide treatment, but not following phenobarbital treatment. In addition, the activity of PROD was increased in fluopicolide-treated females by only about 2-fold, whereas there was an increase of about 12-fold in the corresponding phenobarbital-treated females. Slight decreases in lauric acid hydroxylation were observed in both male and female rats treated with fluopicolide or phenobarbital. In addition, the glucuronidation of 4-nitrophenol was significantly increased to similar extents in male and female rats following treatment with fluopicolide or phenobarbital (Totis, 2008).

No study of cell proliferation in rat liver was available for comparison with the results obtained in mice.

Comparison of the fluopicolide data presented in Tables 30 and 31 suggests that there are conspicuous similarities between the female mice and the male rats, whereas there are fewer similarities between the female mice and female rats. The responses to phenobarbital treatment were generally similar; complete concordance of fluopicolide and phenobarbital data occurs only by chance selection of the dose levels. The fold increases (rounded) for the five parameters were as shown in Table 33.

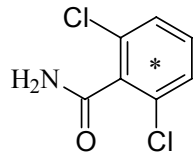
3. Toxicology of metabolites

The following are metabolites on which toxicology has been conducted. M-01 and M-02 are the structures derived when fluopicolide is metabolized to the phenyl and the pyridyl moieties, respectively.

3.1 M-01 (2,6-dichlorobenzamide)

M-01 (AE C653711; BAM; 2,6-dichlorobenzamide) is found in rat liver and on rotational crops. Its chemical structure is shown in Figure 5.

Figure 5. Chemical structure of M-01



(a) Biochemical aspects

Toxicokinetic studies on the absorption, distribution, metabolism and excretion of the metabolite AE C653711 have been performed in Sprague-Dawley CD rats using [phenyl-U-^{14}C]-labelled AE C653711. Following single oral administration of ^{14}C-labelled AE C653711 at doses of 10 and 150 mg/kg bw to male and female rats, radioactivity was eliminated mostly in the urine (approximately 82% of the dose), with low levels eliminated in faeces (approximately 13% of the dose). The rate of elimination was relatively slow.

The highest concentrations in tissues were seen in the kidney and liver after the 10 mg/kg bw dose and in the skin and fur, kidneys and liver after the 150 mg/kg bw dose. Tissue concentrations increased by approximately 5-fold for a 15-fold increase in dose.

Overall, multiple dosing (14 daily doses at 10 mg/kg bw) did not have any significant impact on absorption, distribution, metabolism or elimination compared with results after single oral dosing. Thus, the results in this study showed that the routes and the rates of excretion were maintained despite the multiple dosing, which meant that most of the radioactivity was eliminated via the urinary route. The distribution pattern in the tissues was also similar between single and multiple dosing, with the highest mean concentrations observed in the skin and fur, kidney and liver. Bioretention or accumulation was therefore not indicated.

Biotransformation was similar between dose levels and sexes, consisting of 1) hydrolysis of the amide group; 2) hydroxylation and subsequent conjugation with either glucuronic acid or sulfate and 3) the loss of a chlorine atom, followed by glutathione conjugation and further metabolism of the glutathione group to the mercapturic acid or *S*-methyl metabolites (Gutierrez, 2003b,c,d).

(b) Acute toxicity

M-01 has relatively low acute oral toxicity, with an LD_{50} of 2000 mg/kg bw in male and 500 mg/kg bw in female Wistar rats (Schüngel, 2003a). It is notable that this metabolite is of significantly greater acute oral toxicity than the parent fluopicolide (LD_{50} >5000 mg/kg bw), which is relevant in the risk assessment of this metabolite.

(c) Short-term studies of toxicity

A 13-week toxicity study of M-01 was performed in Wistar rats using dietary concentrations of 0, 50, 180, 600 and 2300 ppm, equal to 0, 4, 14, 49 and 172 mg/kg bw per day in males and females combined. There were no mortalities, and clinical signs were hair loss in females of the 600 and 2300 ppm groups. Reduced body weight gains and food consumption were observed at dose levels of 600 ppm and higher in females and 2300 ppm in males. There was significant reduction in skeletal muscle tone in both sexes at 600 ppm and higher. Blood cell parameters were unchanged by treatment, except for blood coagulation times, which were significantly decreased in both sexes at 2300 ppm. Blood chemistry investigations showed increased protein and cholesterol concentrations in all rats of the 2300

ppm group at the end of the treatment period. Changes in organ weights were not clearly related to treatment, and those changes that were described were not accompanied by microscopic changes. No effects on liver or kidney function were observed.

The NOAEL of M-01 was 180 ppm, equal to 14 mg/kg bw per day, based on reductions in food consumption and body weight gain and reduced skeletal muscle tone at 600 ppm, equal to 49 mg/kg bw per day (Boschman et al., 1967).

In a 90-day oral toxicity study in dogs, groups of four male and four female Beagle dogs (six of each sex in the control group) were administered M-01 (purity 97%; batch No. 133/2/4/104) in the diet at dose levels of 0, 100, 300 or 2000 ppm, equivalent to 0, 7.5, 22.5 and 150 mg/kg bw per day. The study suffered from a number of deficiencies, including ascarid infestation of most dogs, absence of dietary stability and homogeneity data, lack of ophthalmology and histology of many tissues, as well as restricted blood chemistry investigations.

There were no mortalities, but from week 5 until termination, high-dose dogs were observed to have thin appearance, dull coat and hair loss. Mean terminal body weights were slightly decreased in males (6%) and females (8%) at 2000 ppm, but these changes in body weight were not considered to be biologically significant. Food consumption in the treatment groups was reported to be similar to that of controls. No treatment-related changes were observed in haematological or urinalysis parameters. A 66% ($P < 0.01$) increase in serum alkaline phosphatase activity was observed in females at 2000 ppm. No difference in bromsulfthalein plasma half-life (a measure of liver function) was observed between control and high-dose dogs of either sex. Mean liver weights were increased in males by 12%, and mean testes weights were decreased by 22–25%. In females, mean liver weight (adjusted for initial body weight) was increased by 27% ($P < 0.05$) and 35% ($P < 0.01$) at 300 and 2000 ppm, respectively. In view of the increased mean serum alkaline phosphatase activities at 2000 ppm, the increase in mean adjusted liver weight at this dose was considered toxicologically significant.

The NOAEL in this 3-month study of toxicity in dogs is 300 ppm, equivalent to 22.5 mg/kg bw per day, based on clinical signs in males and females and increased liver weight and serum alkaline phosphatase activities in females at 2000 ppm, equivalent to 150 mg/kg bw per day (Walker, 1967).

In a 2-year oral toxicity study in dogs, groups of four male and four female Beagle dogs were administered M-01 (purity >98%; batch No. 232580250) dissolved in acetone and mixed into the diet at concentrations of 0, 60, 100, 180 or 500 ppm, equal to 0, 1.5, 2.5, 4.5 and 12.5 mg/kg bw per day, for approximately 2 years. All groups were observed daily for general health and behaviour, food consumption and growth. Laboratory investigations for haematology and blood chemistry were performed prior to treatment and at approximately 3-month intervals until study termination. Haematological examination comprised haemoglobin content, haematocrit, erythrocyte, leukocyte and differential leukocyte counts, prothrombin time and kaolin-cephalin clotting time. Blood chemistry tests consisted of bromsulfthalein clearance, serum protein concentration, urea, glutamic–pyruvic transaminase (i.e. alanine aminotransferase) activity and plasma alkaline phosphatase activity. All surviving animals were killed at the end of the treatment period and subjected to detailed gross pathology, organ weight analysis and microscopic examination.

The study was performed before GLP requirements and was not quality assured. A 5000 ppm concentrate was initially prepared by mixing a solution of the compound in a minimal quantity of acetone with the appropriate amount of dog food, and various dietary concentrations were prepared from the concentrate. The control diet was also mixed with an equal quantity of acetone. The test concentration in the diet was verified at various intervals during the in-life phase of the study, and the values for the treatment concentrations are considered to be within acceptable deviation of the nominal concentration. Homogeneity and stability of the test substance in the diet were not reported.

Table 34. Summary of the findings on body weight development in the 2-year dietary study with M-01 in dogs

	Body weight (g)				
	Dietary concentration (ppm)				
	0	60	100	180	500
Week 0					
Males	10.7	10.5	10.7	10.9	10.8
Females	9.8	10.4	9.9	9.9	10.0
Week 2					
Males	11.1	11.2	11.0	10.8	11.0
Females	10.3	10.3	10.2	10.1	10.0*
Week 6					
Males	11.9	12.0	11.5	11.4	11.3
Females	11.3	11.1	10.8	10.6	10.2**
Week 15					
Males	12.7	12.5	11.8	12.1	11.6
Females	11.9	11.6	11.5	11.2	10.4**
Week 54					
Males	14.4	14.4	13.6	13.3	12.4
Females	13.7	13.8	12.6	12.4	10.8**
Week 80					
Males	15.2	15.0	14.4	14.0	13.0
Females	14.4	14.4	13.2	12.8	11.3**
Week 104					
Males	15.5	15.5	15.1	14.5	13.6
Females	15.0	15.0	13.7	13.4	11.5**

From Wilson & Thorpe (1971)
* $P \leq 0.05$; ** $P \leq 0.01$

Each dog received 400 g of diet moistened with an equal volume of water during the first year, and this was increased to 600 g in the second year.

General health and behaviour of treated and control dogs were similar throughout the 2-year period. Body weight gain in females of the 500 ppm dose group was statistically significantly reduced throughout the study, and statistically non-significant reductions (approximately 10%) were observed at 180 ppm from week 63 to termination; however, the dogs were gaining weight once more at the end of the study, and the finding is not considered to be adverse. Body weights of males were non-significantly reduced in the 500 ppm group by about 13% from week 54 to the end of the experiment (Table 34). Food consumption data for individual dogs to permit further analysis were not provided.

Haematology and blood chemistry values were similar in controls and treated dogs throughout the study, and occasional changes did not reveal any treatment or dose relationships. At necropsy, increased relative liver weights were noted in 500 ppm group males, whereas reductions in absolute and relative liver and kidney weights were observed in females (Table 35). There was no clear biological relevance for these findings, as no dose–response relationship was evident.

Table 35. Summary of the findings on organ weight changes in the 2-year dietary study with M-01 in dogs

Parameter	Dietary concentration (ppm)				
	0	60	100	180	500
Organ weight (g)					
Liver					
Males	499	531	493	443	544
Females	621	434**	407**	436**	405**
Kidneys					
Males	69.6	75.3	78.9	70.1	67.0
Females	73.1	62.6	57.4*	58.4*	49.8**
Relative organ weight (g/100 g bw)					
Liver					
Males	3.27	3.44	3.24	3.04	4.01*
Females	4.14	2.93**	2.99**	3.24**	3.49**
Kidneys					
Males	0.460	0.486	0.523	0.477	0.487
Females	0.483	0.427	0.422	0.437	0.433

From Wilson & Thorpe (1971)
* $P \leq 0.05$; ** $P \leq 0.01$

Gross pathological and histopathological examination of organs and tissues did not reveal any treatment-related changes.

The NOAEL in the 2-year dietary study with M-01 in dogs was 180 ppm, equal to 4.5 mg/kg bw per day, based on reduction in body weight gain at 500 ppm, equal to 12.5 mg/kg bw per day (Wilson & Thorpe, 1971).

(d) Long-term studies of toxicity and carcinogenicity

In a non-GLP carcinogenicity study, groups of 35 male and 35 female Sprague-Dawley CD rats were fed diets containing 0, 60, 100, 180 and 500 ppm of M-01 (purity unknown; batch No. 133/2/4/104), equal to 0, 2.0, 3.5, 5.7 and 17.6 mg/kg bw per day in males and 0, 2.7, 4.1, 8.6 and 21.3 mg/kg bw per day in females, for 2 years. There were no treatment-related deaths. Body weights were significantly reduced in males and females of the 500 ppm group, and food consumption was slightly reduced in females of the 500 ppm group. Eye examinations of the control and high dose group rats showed no effects of treatment. Blood examination revealed occasional minor depression of red cell parameters (haemoglobin concentration, erythrocyte counts and haematocrit), mainly in males and to a lesser extent in females, at 500 ppm. Blood chemistry and urinalysis did not show any treatment-related changes at the high dose level.

No treatment-related relative or absolute organ weight changes were observed at any dose levels. Gross postmortem examination did not reveal any treatment-related abnormalities.

The only treatment-related histopathological findings were confined to the liver and were observed only in females at 500 ppm (Table 36).

These liver changes were characterized by hepatocyte vacuolation and degeneration as well as fat deposition. In addition, a higher, but not statistically significant, incidence of hepatoma was found in 4 of 20 females subjected to liver histology.

Table 36. Liver histopathology in rats treated with M-01 and killed on week 107

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	60	100	180	500	0	60	100	180	500
No. of rats subjected to liver histology	7	10	10	10	15	8	10	10	10	20
Vacuolation, fat deposition, hepatocyte degeneration	0	1	1	0	1	0	3	0	2	9
Hepatoma	1	0	1	0	1	0	0	0	0	4

From Wheldon et al. (1971)

Table 37. Neoplastic lesions reported from the re-evaluation of livers of rats treated with M-01

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	60	100	180	500	0	60	100	180	500
No. of livers examined	26	28	32	25	34	24	28	27	32	35
Hepatocellular adenoma	1	0	1	0	1	0	1	0	0	5
Hepatocellular carcinoma	2	1	2	1	0	0	0	0	0	0

From Connick, Crome & Gonipath (1996)

While other histopathological findings were observed in some tissues, they occurred in all groups, including controls, and were frequently recorded in rats of this age and strain. They were not considered to be related to treatment (Wheldon et al., 1971).

The microscopic examination of tissues in this study was incomplete, but the tissues had been fixed and stored. In 1996, a new study of these tissues was undertaken, and this revealed a slightly increased incidence of neoplastic hepatocellular adenomas (previously referred to as hepatomas) in female rats in the 500 ppm group in comparison with concurrent controls (0/24 versus 5/35, $P = 0.049$, Fisher's exact test) (Table 37). The hepatocellular adenoma found in one 60 ppm group female was in an individual for which no liver lesion was reported in the original histopathological evaluation. No hepatocellular carcinomas were observed in females at any dose, and no increase in hepatocellular tumours was seen in treated male rats.

Non-neoplastic lesions in the livers observed during this re-evaluation are presented in Table 38.

Increased incidences of eosinophilic (focal and areas) and basophilic (focal and areas) hepatocytes were detected in treated rats from the 100, 180 and 500 ppm M-01 dosage groups. The effects were generally more pronounced in female rats. No effect was seen at 60 ppm M-01. In addition, an increased incidence of vacuolation of centrilobular hepatocytes was detected in both sexes at the 500 ppm dosage level. In some female rats from this dosage group, this change was associated with or masked by the areas or foci of eosinophilic hepatocytes.

Table 38. Non-neoplastic lesions reported from the re-evaluation of livers of rats treated with M-01

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	60	100	180	500	0	60	100	180	500
No. of livers examined	26	28	32	25	34	25	28	28	32	35
Eosinophilic hepatocytes – focal	6	12	17**	11	21**	5	4	7	16*	23**
Eosinophilic hepatocytes – area	1	3	0	2	4	2	2	1	5	18**
Basophilic hepatocytes – focal	7	11	5	6	9	9	10	6	14	23*

From Connick, Crome & Gonipath (1996)
* $P < 0.05$; ** $P < 0.01$ (Fisher's exact test)

The NOAEL was 60 ppm, equal to 2.0 mg/kg bw per day, based on body weight reductions, increased incidences of eosinophilic and basophilic foci and fat deposition and cellular degeneration in the livers at 100 ppm, equal to 3.5 mg/kg bw per day (Connick, Crome & Gonipath, 1996).

(e) Genotoxicity

The genotoxicity profile of M-01 was assessed in bacterial gene mutation assays with *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 and (in one assay) TA102 strains at doses up to 5000 µg/plate (Koorn, 1992; Herbold, 2003b), a V79 cell *hprt* gene locus assay at doses up to 5000 µg/ml (Herbold, 2003c), an in vitro rat liver cell unscheduled DNA synthesis assay at doses up to 1000 µg/ml for 18 h (van de Waart, 1993a) and a mouse bone marrow cell micronucleus assay in vivo at a single oral dose of 250 mg/kg bw and cell sampling after 24, 48 and 72 h (van de Waart, 1993b). No evidence for genetic toxicity or mutagenicity emerged from any of these assays.

(f) Reproductive toxicity

In a three-generation study of reproduction in rats, groups of 10 male and 20 female Long-Evans rats were administered M-01 (purity 99.5%; batch No. 195) in the diet at concentrations of 0, 60, 100 and 180 ppm, equal to 0, 4.5, 7.5 or 13.5 mg/kg bw per day, throughout the three generations. Each generation of parents was mated twice to produce two litters. The F_0 generation rats were administered M-01 in the diet for 79 days before mating when they were 100 days old. A 2-week period was allowed for the mating of each female, with males rotated once during that time. All pups from the first litters, F_{1A}, were discarded at weaning, and the parent rats were mated again after 10 days to produce the F_{1B} generation. From this generation, pups were randomly selected, after exclusion of runts, maintained on the same diets as their respective F_0 parents and mated in turn when 100 days old to produce the F_{2A} and F_{2B} litters. Randomly selected F_{2B} rats were allowed to mate when 100 days old to produce the F_{3A} and F_{3B} litters.

The numbers of pups in each litter were counted on the day of birth and on the fifth day. Litters greater than 10 were reduced to 10 on the fifth day. On day 21, the weanlings were counted and weighed and either sacrificed or saved for continuation on the diets. Parent rats were weighed, sacrificed and examined grossly when no longer required. Ten male and 10 female F_{3B} weanlings from the control and 180 ppm dose groups and 5 males and 5 females from the 60 and 100 ppm dose groups were selected for necropsy. Individual body weights and brain, liver and kidney weights were recorded. Sections of brain, heart, lung, liver, spleen, kidney and testes were preserved for histological examination.

Table 39. Fertility index[a] of rat groups in the three-generation study of M-01

Generation	Parameter	Dietary concentration (ppm)			
		0	60	100	180
F_{1A}	Ratio	17/20	18/20	20/20	20/20
	Index	85	90	100	100
F_{1B}	Ratio	20/20	18/20	18/20	19/19
	Index	100	90	90	100
F_{2A}	Ratio	18/20	19/20	20/20	20/20
	Index	90	95	100	95
F_{2B}	Ratio	18/20	20/20	20/20	20/20
	Index	90	100	100	100
F_{3A}	Ratio	19/20	20/20	20/20	20/20
	Index	95	100	100	100
F_{3B}	Ratio	19/20	20/20	19/20	20/20
	Index	95	100	95	100

From Hine, Eisenlord & Loquvam (1971)

[a] Fertility index = number of pregnancies/number of matings × 100.

Statistical analysis was performed on the numbers of pups, their survival at 21 days, body weights of weanlings at 21 days, weights of parental rats when they were killed and relative organ weights of F_{3B} weanlings. Dunnett's test was applied for the comparison of treated and control rats. Fertility, gestation, viability and lactation indices were calculated from litter production and mortality and tested for significance using the chi-squared test. The study was performed before GLP regulations were published. The study sponsor has observed that the study is of poor quality and presents several deficiencies, including the absence of any individual data, the absence of measurement of several parameters to evaluate the reproductive performance (mating index, precoital time, pregnancy rate, length of gestation) and the absence of fetal body weight evolution data from birth up to weaning. It will not be possible to obtain such data retrospectively, given that the laboratory that conducted the work is no longer in existence. Nevertheless, the study is acceptable with the proviso that the limitations mentioned are considered in its assessment. A significant range of core reproductive toxicity parameters was investigated. A new study is considered unnecessary, as there are no indications for further concern.

There were no treatment-related signs of toxicity or differences in behaviour and appearance of the rats during the study. Hyperexcitability was noted in a few pups of the F_{1B} generation only and is not considered treatment related. Fertility was not affected by the treatment and ranged from 85% to 100% throughout the study, with no significant intergroup differences (Table 39).

The average number of pups per litter in the treated and control groups did not show any treatment-related intergroup differences and ranged from 9.3 to 12.2 in the first generation, from 8.7 to 10.4 in the second generation and from 9.1 to 11.2 in the third generation (Table 40).

The investigators reported that the mean percentages of surviving pups between days 1 and 21, when analysed by the Dunnett's test, were similar in all groups and in all generations and did not show any treatment-related differences from controls. Gestation indices were similar in all dose groups and in all generations (Table 41).

Viability indices showed occasional statistically significant differences for the number of pups alive at day 5; however, these differences did not show any dose–response or treatment relationship. Although a treatment relationship cannot be ruled out for the observation for the F_{3B} 180 ppm dose

Table 40. Summary of litter sizes at birth and mean per cent survival (day 21) for all dose groups in the three-generation study of M-01 in rats

Generation	Dietary concentration (ppm)							
	0		60		100		180	
	Litter size	% survival	Litter size	% survival	Litter size	% survival	Litter size	% survival
F_{1A}	10.5	72.3	9.9	74.2	10.6	76.1	9.3	69.1
F_{1B}	10.6	63.6	12.2	68.8	11.0	60.7	11.3	75.0
F_{2A}	8.8	90.8	9.5	94.7	8.7	95.1	9.6	95.0
F_{2B}	9.4	89.5	10.2	94.4	10.0	97.7	10.4	86.5
F_{3A}	9.1	80.0	11.2**	80.5	10.6	80.3	10.8*	82.6
F_{3B}	9.8	85.4	11.2	89.8	10.5	83.9	10.2	84.0

From Hine, Eisenlord & Loquvam (1971)
* $P = 0.05$; ** $P = 0.01$

Table 41. Gestation index[a] of rat groups in the three-generation study of M-01

Generation	Parameter	Dietary concentration (ppm)			
		0	60	100	180
F_{1A}	Ratio	17/17	18/18	20/20	20/20
	Index	100	100	100	100
F_{1B}	Ratio	20/20	18/18	18/18	19/19
	Index	100	100	100	100
F_{2A}	Ratio	18/18	19/19	20/20	19/19
	Index	100	100	100	100
F_{2B}	Ratio	18/18	20/20	20/20	20/20
	Index	100	100	100	100
F_{3A}	Ratio	19/19	20/20	20/20	20/20
	Index	100	100	100	100
F_{3B}	Ratio	19/19	20/20	20/20	20/20
	Index	100	100	100	100

From Hine, Eisenlord & Loquvam (1971)
[a] Gestation index = number of litters with live pups/number of pregnancies × 100.

group, it has to be considered against the absence of any significant effect in five other groups of litters from the other generations and the total numbers of pups alive in the 180 ppm group (Table 42).

Mean body weights of weanlings were significantly lower for the F_{3A} and F_{3B} 180 ppm dose groups by 12% and 14%, respectively, and the reductions are considered to be biologically relevant. Significantly lower body weights were also observed in the F_{1B} generation at the 60 and 180 ppm doses by 14% and 15%, respectively, but there was no dose-related response (Table 43).

The mean terminal body weight was statistically significantly lower by 6% ($P < 0.05$) for the 180 ppm F_{2B} females compared with the concurrent control (Table 44). This small change is

Table 42. Viability[a] and lactation[b] indices of rat groups in the three-generation study of M-01

Generation	Parameter	Dietary concentration (ppm)			
		0	60	100	180
Viability					
F_{1A}	Ratio	172/178	171/179	190/212	180/180
	Index	96.6	95.5	89.6**	96.8
F_{1B}	Ratio	197/213	198/219	181/198	207/214
	Index	92.5	90.4	91.4	96.7
F_{2A}	Ratio	150/158	177/180	167/174	172/182
	Index	94.9	98.3	96.0	94.5
F_{2B}	Ratio	161/170	202/205	194/200	186/207
	Index	94.7	98.5*	97.0	89.9
F_{3A}	Ratio	164/172	219/224	205/211	194/215
	Index	95.3	97.8	97.2	90.2
F_{3B}	Ratio	178/186	217/223	190/199	175/203
	Index	95.7	97.3	95.5	86.2**
Lactation					
F_{1A}	Index	73.6	74.7	83.3*	66.9
F_{1B}	Index	63.3	70.9	63.8	73.7*
F_{2A}	Index	96.5	96.4	96.8	98.2
F_{2B}	Index	96.7	95.1	98.9	95.7
F_{3A}	Index	86.8	82.4	84.0	89.9
F_{3B}	Index	93.3	90.2	86.3*	94.0

From Hine, Eisenlord & Loquvam (1971)
* $P \leq 0.05$; ** $P \leq 0.01$
[a] Viability index = number of pups alive at 5 days/number of pups born × 100.
[b] Lactation index = number of live pups on day of examination/number of pups at day 5 after culling × 100.

Table 43. Mean body weight of weanlings of rat groups in the three-generation study of M-01

Generation	Mean body weight (g)			
	Dietary concentration (ppm)			
	0	60	100	180
F_{1A}	38.7	38.0	37.9	41.0
F_{1B}	38.9	33.4*	35.1	33.1*
F_{2A}	34.4	32.6	32.8	32.7
F_{2B}	38.6	35.5	36.7	36.8
F_{3A}	35.0	32.9	34.4	30.9*
F_{3B}	36.1	33.8	35.3	31.1**

From Hine, Eisenlord & Loquvam (1971)
* $P = 0.05$; ** $P = 0.01$

Table 44. Mean terminal body weight of parents of rat groups in the three-generation study of M-01

Generation	Sex	Mean terminal body weight (g)			
		Dietary concentration (ppm)			
		0	60	100	180
F_0	Male	488	499	488	481
	Female	339	328	331	319
F_{1B}	Male	488	499	465	461
	Female	339	317	323	320
F_{2B}	Male	490	487	456	466
	Female	325	322	309	305*

From Hine, Eisenlord & Loquvam (1971)
* $P = 0.05$

Table 45. Mean organ weights and relative organ weight to body weight ratios of F_{3B} weanlings in the three-generation study of M-01

Parameter	Sex	Dietary concentration (ppm)			
		0	60	100	180
Absolute kidney weight (g)	Male	0.434	0.457	0.475	0.450
	Female	0.435	0.470	0.467	0.487
Relative kidney weight ratio (× 100)	Male	1.24	1.31	1.34	1.28
	Female	1.26	1.34	1.33	1.41**
Absolute liver weight (g)	Male	1.42	1.55	1.53	1.56
	Female	1.46	1.61	1.62	1.62
Relative liver weight ratio (× 100)	Male	4.05	4.44	4.33	4.46*
	Female	4.24	4.60	4.62*	4.65**

From Hine, Eisenlord & Loquvam (1971)
* $P = 0.05$; ** $P = 0.01$

considered to have little toxicological significance. In addition, however, the relative consistency of the body weights for the adult female rats removes a possible rationale for the body weight deficits described for the weanling rats that had been nursed by them.

Liver weights relative to body weights of F_{3B} weanlings were statistically significantly increased in females at dose levels of 100 ppm and above and in males at 180 ppm. Considering the significance of the increase at 100 ppm, 1 of 5 females of the 100 ppm dose group had an absolute liver weight greater than the range of the concurrent controls, whereas a second female had an absolute liver weight that was the same as the highest weight of the control group. For the relative liver weight ratio, 3 of 5 animals were clearly outside the range for the control group of 10 animals. This appeared to be the only significant finding at this dose level, but a significant treatment relationship cannot be eliminated in the absence of the investigation of histopathology of the liver. Kidney weight relative to body weight was also increased for the F_{3B} 180 ppm females (Table 45).

The NOAEL for parental toxicity was 180 ppm, equal to 13.5 mg/kg bw per day, the highest dose tested. The NOAEL for reproductive toxicity was 180 ppm, equal to 13.5 mg/kg bw per day, the highest dose tested, based on the absence of reproductive toxicity. The NOAEL for fetal toxicity was 100 ppm, equal to 7.5 mg/kg bw per day, based on effects on mean liver weights relative to body

weights in offspring at 180 ppm, equal to 13.5 mg/kg bw per day (Hine, Eisenlord & Loquvam, 1971).

In a developmental toxicity study, groups of 16 mated female New Zealand White rabbits were administered M-01 (purity 99.4%) by gavage in 1% gum tragacanth at dose levels of 0, 10, 30 or 90 mg/kg bw per day (dose volume 2 ml/kg bw) from day 7 to day 19 of gestation. Rabbits were examined twice daily for mortality and clinical signs of toxicity. Body weights were recorded on days 0, 7, 13, 19, 23 and 28 of gestation, and food consumption was recorded from days 0 to 7, 7 to 13, 13 to 19, 19 to 23 and 23 to 28 of gestation. All surviving rabbits were killed on day 28 of gestation. At postmortem examination, the dams were examined for gross changes and the outcome of pregnancy. The ovaries and uteri were removed and examined, and the following data were recorded: number of corpora lutea and number and intrauterine position of implantations, subdivided into live fetuses, early intrauterine deaths and late intrauterine deaths.

Fetuses were examined for external, visceral and skeletal abnormalities. Fetal abnormalities were recorded as major (rare and/or possibly lethal), minor (deviations from normal that were common at either gross necropsy or skeletal examination) or variants (retarded sternebral or phalangeal ossification, asymmetric insertion of the pelvic girdle or the presence of supernumerary ribs). The data were subjected to statistical analysis. The study was certified to comply with GLP regulations and subjected to a quality assurance audit. Dose levels were reportedly based on two range-finding studies in rabbits, which were not submitted. The analysis of the dosing formulations showed that the actual concentrations were close to the nominal values.

Three 90 mg/kg bw per day dose group females were killed following abortion on day 19, 21 or 22 of gestation. Maternal toxicity was observed at 90 mg/kg bw per day. A further two dams were killed when found in a moribund condition following body weight loss, thin appearance and reduced food intake in the majority of these animals during the dosing period. No consistent treatment-related macroscopic changes were observed at postmortem examination.

In the 30 mg/kg bw per day dose group, two females were killed when found in a moribund condition on days 12 (without prior clinical signs recorded) and 14 (fur staining and iris problems noted before dosing began, but thin on days 11–13) of gestation. Although a treatment relationship could not be entirely excluded, it was considered unlikely, as mortality was also observed in the 0 and 10 mg/kg bw per day groups. These consisted of one rabbit that aborted on day 23 (fur staining noted before dosing began) in the 10 mg/kg bw per day group, one rabbit that was killed because of its moribund condition on day 20 (thin on days 18–20) and one rabbit that aborted on day 24 (fur staining noted before dosing began). No consistent, treatment-related findings were recorded at necropsy in these rabbits. The occurrence of these deaths relatively late in the study suggests that perhaps they should not contribute to considerations for an acute reference dose (ARfD). However, it is noted that the pattern of reduced food consumption and thin appearance (reported for at least one of the two deaths at 30 mg/kg bw per day) is consistent for deaths and moribund condition in all groups. Rabbits that aborted also showed lower body weights, reduced food intake or fur staining, and there appears to be no clear basis for the exclusion of a treatment relationship for the 30 mg/kg bw per day dose group other than on a comparison of incidences. The summary of body weights and food consumption across dose groups suggests that toxicity resulting in effects on body weight and food consumption was apparent only in the top dose group (Table 46).

There was a marked decrease in group mean body weight of females in the 90 mg/kg bw per day dose group during the early part of the dosing period, with 9 out of the 11 surviving does losing weight between days 7 and 13 of gestation. Body weight gain of these animals from day 13 of gestation up to the end of the dosing period was reduced compared with that of the control (Table 46). The body weight gain of the females in the 10 and 30 mg/kg bw per day dose groups throughout gestation was similar to that of the controls. There was a marked reduction in food

Table 46. Summary of mean food intake and body weight in the developmental toxicity study in rabbits given M-01

Parameter	Dose level (mg/kg bw per day)			
	0	10	30	90
Mean food intakes (g/day)				
- Days 0–7	184	183	190	190
- Days 7–13	189	200	192	94***
- Days 13–19	196	199	198	102***
- Days 19–23	177	181	186	203
Mean total food intake (g/period)				
- Days 0–28	4980	5015	5053	4266
Mean body weight (kg)				
- Day 7	3.74	3.65	3.73	3.80
- Day 19	4.02	3.95	4.01	3.72
- Day 28	4.17	4.04	4.11	3.99
% body weight change				
- Day 7–19	7.5	8.2	7.5	−2.1***

From McIntyre (1986)
*** $P < 0.001$

intake of the 90 mg/kg bw per day dose group rabbits throughout the dosing period. Some compensatory increase in food consumption was observed after cessation of dosing on day 19 of gestation. The majority of the 90 mg/kg bw per day dose group females had a thin appearance. Fur staining was also common, but it was present in most rabbits before dosing began and so is unrelated to treatment. These observations continued even after cessation of dosing on day 19 of gestation. The clinical condition of the females in the 10 and 30 mg/kg bw per day dose groups was comparable with that of the controls and was restricted to those changes that are not uncommon in this strain of rabbit in these laboratories.

There were no treatment-related maternal macroscopic postmortem findings on day 28 of gestation. Pregnancy incidence was between 93.8% and 100% in all groups (Table 47). The mean number of corpora lutea and implantations and the extent of preimplantation loss showed some intergroup variations, but there were no dose–response relationships in the frequencies of these parameters. Postimplantation loss and subsequent litter size were not adversely affected by treatment. Mean fetal weight was slightly (5.8%), but not statistically significantly, reduced in the 90 mg/kg bw per day dose group.

The overall incidence of fetuses with major malformations was not adversely affected by treatment. An increase in the number of malformed fetuses was observed in the 10 mg/kg bw per day group, but these findings were isolated and not observed at higher dose levels. The overall type and incidence of minor external and visceral defects and variants showed some intergroup variations, but no consistent treatment-related trends were apparent (Table 47). Ossification parameters were similar to the background controls and not considered to be adversely affected by treatment.

The NOAEL for maternal toxicity was 30 mg/kg bw per day, based on maternal deaths and increased incidence of abortions, most likely a consequence of body weight loss, at 90 mg/kg bw per day. The NOAEL for fetotoxicity was 30 mg/kg bw per day, based on a statistically non-significant reduction in birth weights at 90 mg/kg bw per day. M-01 was not teratogenic in this developmental

Table 47. Summary of reproductive and fetal findings in the development toxicity study in rabbits given M-01

Parameter	Dose level (mg/kg bw per day)			
	0	10	30	90
Number of pregnant females	16/16	16/16	16/16	15/16
Number of pregnant females at 28 days	14	15	14	11
Mean number of corpora lutea per female	10.9	9.7	10.1	10.3
Mean number of implantations per female	10.4	9.0	8.4	9.5
% preimplantation loss	4.6	7.5	16.3	8.0
Mean number of early intrauterine deaths per female	1.3	0.5	0.1	0.5
Mean number of late intrauterine deaths per female	0.4	0.2	0.4	0.3
% postimplantation loss	16.6	7.4	6.8	8.7
Mean number of fetuses per female	8.6	8.3	7.9	8.6
% of implantations	83.4	92.6	93.2	91.3
Mean fetal weight (g)	36.0	36.4	36.8	33.9
External and visceral defects				
Number showing major defects	0	2	0	0
% of fetuses examined	0	1.6	0	0
Number showing minor defects	11	16	17	14
% of fetuses examined	9.1	12.8	15.5	14.7
Number showing variants	21	23	11	26
% of fetuses examined	17.4	18.4	10	27.4
Skeletal defects				
Number showing major defects	2	7	2	2
% of fetuses examined	1.7	5.6	1.8	2.1
Number showing minor defects	34	43	44	37
% of fetuses examined	28.1	34.4	40.0	38.9
Number showing variants	101	101	91	78
% of fetuses examined	83.5	80.8	82.7	82.1
Total number of major defects	2	7	2	2
% of fetuses examined	1.7	5.6	1.8	2.1

From McIntyre (1986)

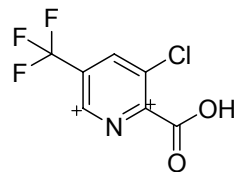
toxicity study in rabbits, and the NOAEL for developmental toxicity was 90 mg/kg bw per day, the highest test dose (McIntyre, 1986).

In conclusion, these data showed that the toxicological profile of the metabolite M-01 is similar to that of fluopicolide; the NOAELs tend to be lower for the metabolite.

3.2 M-02 (3-chloro-5-(trifluoromethyl)pyridine-2-carboxylic acid)

The chemical structure of M-02 (AE C657188; PCA; 3-chloro-5-(trifluoromethyl)pyridine-2-carboxylic acid), which is found in rat urine, is shown in Figure 6.

Figure 6. Chemical structure of M-02



A toxicokinetic study on the absorption, distribution, metabolism and excretion of the metabolite M-02 has been performed in Sprague-Dawley CD rats using [pyridyl-2,6-^{14}C]-labelled M-02 at a single oral dose of 10 mg/kg bw. The rate of elimination was rapid for both male and female rats, with at least 90% of the total administered radioactivity eliminated within the first 48 h after dosing. The total recovery in urine accounted for a mean of 78.5% of the dose, with a further 8.2% being found in the cage wash. Lower levels were found in the faeces, the total recovery accounting for a mean of 6.6% of the dose. No pulmonary excretion was detected. The estimated minimum mean level of absorption was calculated to be 87%. Thus, this metabolite of fluopicolide demonstrated high oral bioavailability and low potential for bioaccumulation. No sex difference was observed in terms of the routes and rates of elimination. There was little remaining radioactivity in the tissues. Radioactivity was detected only in the residual carcass and in the skin and fur. The metabolism investigations showed that the administered metabolite, M-02, was the major radioactive component excreted in urine and faeces (Gutierrez, 2002).

M-02 has very low acute toxicity, with an oral LD_{50} value of >2000 mg/kg bw in rats (Coleman, 2000).

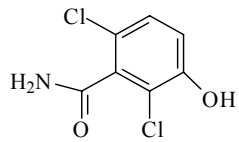
The genotoxicity profile of M-02 was assessed in a bacterial gene mutation assay with *S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli uvr*A/pKM101 at doses up to 5000 µg/plate (Kitching, 2000), a V79 cell *hprt* gene locus assay at doses up to 5000 µg/ml (Herbold, 2003d) and a chromosomal aberration assay with human peripheral blood lymphocytes at doses up to 2256 µg/ml for 3 h in the presence of S9 and 723 µg/ml for 20 h in the absence of S9 (Lloyd, 2003a,b). No evidence for genetic toxicity or mutagenicity emerged from any of these assays.

A 28-day toxicity study was performed in groups of five male and five female Sprague-Dawley rats administered M-02 in the diet at concentrations of 0, 20, 200, 2000 and 20 000 ppm, equal to 0, 1.5, 15, 149 and 1574 mg/kg bw per day in males and 0, 1.63, 15.9, 162 and 1581 mg/kg bw per day in females. There were no reports of mortalities or clinical signs of toxicity. Food consumption and mean body weights were unaffected by treatment in male rats, whereas there were small reductions in both parameters among females of the 20 000 ppm group. Examination of the rat eyes showed no abnormalities, and there were no toxicologically significant changes in blood cell parameters. Plasma phosphate concentrations in male rats were reduced by 12% in comparison with the controls at 20 000 ppm. Urinalysis was unaffected by treatment. No treatment-related change was seen in mean terminal body weights, mean absolute and relative organ weights, or gross postmortem or microscopic examination in rats of the 20 000 ppm group. The NOAEL of M-02 was 20 000 ppm, equal to 1574 mg/kg bw per day, the highest dose tested (Kennel, 2001). These data showed that the metabolite M-02 is less toxic than the parent fluopicolide.

3.3 M-04 (2,6-dichloro-3-hydroxybenzamide)

M-04, or 2,6-dichloro-3-hydroxybenzamide, is found on rotational crops. Its chemical structure is shown in Figure 7.

Figure 7. Chemical structure of M-04



M-04 has very low acute toxicity, with an oral LD_{50} value of >2000 mg/kg bw in rats (Schüngel, 2003b).

The genotoxicity profile of M-04 was assessed in three in vitro and two in vivo assays. No evidence for mutagenicity of M-04 was observed in a bacterial reverse mutation assay with *S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 at doses up to 5000 µg/plate (Herbold, 2003e) or a V79 cell *hprt* gene locus assay at doses up to 4400 µg/ml (approximately 20 mmol/l) (Herbold, 2003f). In an in vitro chromosomal aberration assay performed in human lymphocytes, no significant effects were observed following exposure to M-04 at concentrations up to 2060 µg/ml for 3 h in either the presence or absence of S9. A small, but significant, increase in chromosomal aberration frequency was observed after 20 h of exposure to M-04 at concentrations between 1159 and 2060 µg/ml (the whole range) in the absence of S9. Although there were substantial numbers of cells with aberrations, assessed both including and excluding gaps, the vehicle control value was also very much higher than in the other two segments of the study. In two segments of the study, there were 4 and 5 cells with aberrations in 200 scored, whereas in the segment in which the significant response was observed, there were 20 cells with aberrations in 200 scored. It is also noted that the untreated culture in the same segment of the experiment did not show a particularly increased number of cells with aberrations excluding gaps (7/200 cells) (Kumaravel, 2003). In an in vivo rat liver unscheduled DNA synthesis assay (Brendler-Schwaab, 2004) and an in vivo mouse bone marrow cell micronucleus assay (Herbold, 2003g), there was no evidence of genetic toxicity or mutagenicity. In both of these in vivo assays, the highest doses induced cytotoxicity in the target organs. It is concluded that M-04 is unlikely to be a genetic hazard for exposed people.

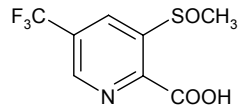
A 28-day toxicity study was performed in groups of 10 male and 10 female Wistar rats administered M-04 in the diet at concentrations of 0, 20, 200, 2000 and 20 000 ppm, equal to 0, 1.6, 16.2, 159.2 and 1775.0 mg/kg bw per day in males and 0, 2.1, 20.4, 230.6 and 1930.8 mg/kg bw per day in females. There were no dose-related mortalities or clinical signs of toxicity and no indications of neurotoxicity from an FOB. Although there were no effects of treatment on food intake, body weight and body weight gain were reduced in rats of the 20 000 ppm dose group. In male rats of that group, at termination, body weights were 10% lower than in the controls. There were no treatment-related effects on the eyes. Observations on blood showed that in the 20 000 ppm group, there was a significantly reduced haemoglobin concentration in females, a significantly higher platelet count in both sexes and a significantly increased concentration of cholesterol. The target organs identified for toxicity were the liver and the kidneys at 20 000 ppm. Liver weights were significantly higher in both sexes, and, in females, there was a minimal cytoplasmic change in peripheral hepatocytes that was accompanied by a reduction in periportally stored fat. Urine volume was reduced and density increased, whereas kidney weights were increased and there was an increased incidence of basophilic cortical tubule cells. Also at 20 000 ppm, thyroids showed a granular or clumpy alteration of the follicular colloid in both sexes; there was also a flattening of the follicular epithelium indicative of reduced thyroid function in 2 of 10 males (Eiben & Rinke, 2003). The NOAEL of M-04 was 2000 ppm, equal to 159.2 and 230.6 mg/kg bw per day in males and females, respectively, based on lower body weights, reduced haemoglobin concentration, increased plasma cholesterol concentration, increased liver and kidney weights and histological findings in the liver, kidney and thyroid at 20 000 ppm, equal to 1775 and 1931 mg/kg bw per day in males and females, respectively.

In conclusion, the data showed that the metabolite M-04 is less toxic than the parent fluopicolide.

3.4 M-05 (3-(methylsulfinyl)-5-(trifluoromethyl)pyridine-2-carboxylic acid)

M-05, or 3-(methylsulfinyl)-5-(trifluoromethyl)pyridine-2-carboxylic acid, is found on rotational crops. Its chemical structure is shown in Figure 8.

Figure 8. Chemical structure of M-05



M-05 was shown to be of very low acute toxicity, with an oral LD_{50} of >5000 mg/kg bw in rats (Schüngel, 2003c).

The genotoxicity profile of M-05 was assessed in a bacterial gene mutation assay with *S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 at doses up to 5000 µg/plate (Herbold, 2003h), a V79 cell *hprt* gene locus assay at doses up to 2400 µg/ml (Herbold, 2003i) and a chromosomal aberration assay with human peripheral blood lymphocytes at doses up to 2532 µg/ml for 3 h in the presence of S9 and for 20 h in the absence of S9 (Lloyd, 2003b). No evidence for genetic toxicity or mutagenicity emerged from any of these assays.

A 28-day toxicity study was performed in groups of 10 male and 10 female Wistar rats administered M-05 in the diet at concentrations of 0, 20, 200, 2000 and 20 000 ppm, equal to 0, 1.6, 16.2, 159.2 and 1775.0 mg/kg bw per day in males and 0, 2.1, 20.4, 230.6 and 1930.8 mg/kg bw per day in females. There were no mortalities during the study. Clinical signs considered to be treatment related occurred at 20 000 ppm, but not at lower dose levels. They included scabs around the nose and head region observed in three males, together with soiling around the eye in one male and chromodacryorrhoea in two other males. One female had anogenital soiling. No treatment-related clinical signs were observed at lower dose levels. Neurotoxicity assessment did not reveal any treatment-related effects at any dose level. Significant reductions in food consumption and body weights occurred at 20 000 ppm, but not at lower dose levels. Haematology did not reveal any dose-related effects, and blood chemistry revealed a 10% reduction in plasma organic phosphorus concentration at 20 000 ppm at the end of the study. Coarse casts were found in the urine of 1 of 10 males and 9 of 10 females. There were also slight reductions in urinary pH in males and an increase in urinary volume in both sexes. Liver weights were increased in males at 20 000 ppm, but there was no accompanying hepatic microscopic change. Single-cell necrosis of minimal to moderate severity and minimal to moderate renal tubule degeneration and regeneration were observed in 8 of 10 female rats at 20 000 ppm. The NOAEL was 2000 ppm, equal to 152 mg/kg bw per day in males and 167 mg/kg bw per day in females, based on clinical signs, reductions in body weight and food consumption and renal effects at 20 000 ppm, equal to 1775 mg/kg bw per day in males and 1931 mg/kg bw per day in females (McElligot, 2003).

In conclusion, the data showed that the metabolite M-05 is less toxic than the parent fluopicolide.

4. Observations in humans

As fluopicolide is a new compound, there is little information on exposure of workers and no information on exposure of the general population to fluopicolide. Fluopicolide has been produced so far at pilot scale or formulated by Bayer Crop Science in Lyon (France) and in Dormagen (Germany).

There have to date been no reported incidents of adverse reactions during the pilot-scale manufacture or formulation of fluopicolide.

Comments

Biochemical aspects

In rats given [^{14}C]fluopicolide labelled in either the pyridyl or phenyl ring as a single oral dose at 10 or 100 mg/kg bw, the radiolabel was moderately rapidly absorbed from the gastrointestinal tract (about 70% and 85% of the pyridyl and phenyl labels, respectively). Based on the results of one study of biliary excretion and only for the single dose at 10 mg/kg bw, the extent of oral absorption was 80% for the phenyl radiolabel and 62% for the pyridyl radiolabel. However, blood and plasma kinetic data show that systemic exposure was similar for both radiolabels and for males and females. The bioavailability of the radiolabel, taking into account the material undergoing enterohepatic recirculation, was calculated to be 75–88% of the administered dose. The T_{max} calculated from plasma concentrations was 7–10 h. There were no significant differences related to sex, high or low doses or single versus multiple doses.

Distribution investigated by dissection and liquid scintillation counting methods and confirmed by whole-body autoradiography demonstrated that the highest concentrations of radiolabel were in the liver and kidney and, to a lesser extent, in spleen and blood. Tissue concentrations of radiolabel were consistently low and ranged from 0.46% to 1.25% of the administered dose for the single-dose studies, with a mean of 0.38% for the repeated-dose study.

Elimination from tissues was moderately rapid, such that most radioactivity was eliminated within 48 h after dosing; a subsequent slower terminal elimination phase had a mean half-life of about 103 h. Excretion of the 10 and 100 mg/kg bw oral doses was extensive for pyridyl (69–72%) and for phenyl (82–88%) ring radiolabels and was mainly in the faeces. More than 70% of the administered dose was eliminated within 24 h, but the rate of excretion was low thereafter. Extensive biliary excretion (90%) was demonstrated in bile duct–cannulated rats, in which there was also evidence for enterohepatic recirculation. There was a tendency towards a higher urinary excretion of the pyridyl radiolabel (approximately 20% for the dose at 10 mg/kg bw) compared with the phenyl radiolabel (approximately 10% for the dose at 10 mg/kg bw). This suggests that a proportion of the metabolites that were formed differed between the two radiolabels and were presumably linked to the hydrolysis of the amido group and the formation of M-02 from the pyridyl ring moiety and M-01 from the phenyl ring.

Fluopicolide was extensively metabolized in the rat. The formation of the metabolites M-01 and M-02, which are also residues in plants, was confirmed during the course of the biotransformation investigations. Generally, the biotransformations observed included aromatic ring hydroxylation, hydrolysis, dealkylation, acetylation, oxidative *N*-dealkylation and conjugation with glucuronic acid, sulfate and glutathione. The glutathione conjugates were further metabolized by loss of glycine and glutamic acid to leave cysteine conjugates. The cysteine conjugates were further metabolized either by acetylation to form the mercapturic acids or by carbon–sulfur cleavage followed by *S*-methylation to form *S*-methyl metabolites. The *S*-methyl metabolites were oxidized to both sulfones and sulfoxides.

Toxicological data

The acute toxicity of fluopicolide is low, the oral LD_{50} being >5000 mg/kg bw in rats. Signs of toxicity at this high dose included piloerection within 1–2.5 h after dosing in all rats. Later on day 1, piloerection was accompanied only by hunched posture and abnormal gait. Recovery was complete

by day 3. The acute dermal LD_{50} of fluopicolide in rats was >5000 mg/kg bw. The 4 h acute inhalation LC_{50} of fluopicolide in rats was >5.16 mg/l air (the mean achieved concentration). Fluopicolide was not irritating to rabbit skin and was only transiently slightly irritating to the rabbit eye. Fluopicolide was not a skin sensitizer in the Magnusson and Kligman test in guinea-pigs.

Short-term studies of toxicity with fluopicolide have been performed in mice, rats and dogs. The liver was consistently identified as a target organ in short-term studies in mice, rats and dogs, and the kidney was also a target in male and female rats given higher doses. Increased liver weights were observed in mice, rats and dogs in 28-day and 3-month studies and in dogs in a 1-year study. Microscopic changes observed in the liver included centrilobular hepatocyte hypertrophy in mice and rats and an increased incidence and severity of granulated lymphocytes in rats in the 28-day dietary study. Plasma cholesterol concentrations were increased in rats in the 28-day and 3-month studies and in female dogs in the 1-year study, but they were reduced in mice in the 3-month study. Serum albumin concentrations were also reduced in mice in the latter study. The Meeting considered that these observations were suggestive of impairment of hepatic function at high doses. Renal effects were observed only in rats and consisted of kidney weight increases in males at 28 days and at 3 months and histopathological changes (accumulation of hyaline droplets, single-cell necrosis in the proximal tubule epithelium and small foci of basophilic tubules and granular casts) in males and females at 3 months. Reversibility of the hepatic effects, but not the renal effects, was demonstrated in rats after 3 months of exposure followed by a 28-day recovery period. Other observations made in these short-term studies were restricted to a particular species, sex or treatment duration. They included treatment-related reductions in haemoglobin and erythrocyte volume fraction in male rats and increased urine volume and specific gravity and spleen weight in female rats in the 3-month study.

The NOAELs derived from short-term studies in which fluopicolide was administered orally were between 7 and 17 mg/kg bw per day in mice and rats, and the overall oral NOAEL in dogs was 70 mg/kg bw per day.

In long-term dietary studies in mice and rats, the primary target organs were the liver and kidney. In mice, liver weights were increased at 400 ppm and 3200 ppm in males after 1 year and in males and females after 18 months. Liver masses and nodules were also increased at these doses after 18 months. High incidences of centrilobular hepatocyte hypertrophy were recorded at 400 ppm and 3200 ppm in male and female mice after 18 months. Foci of altered hepatocytes (eosinophilic foci) and hepatocellular adenoma were increased in male and female mice at 3200 ppm, but there was no increase in the occurrence of hepatocellular carcinoma. In rats, liver weights were increased only in males at the highest dose at 2 years, and, microscopically, there was a dose-related increase in the incidence and severity of centrilobular hepatocyte hypertrophy, again in males, at both 1 year and 2 years. No cytochrome P450–related enzymes were measured in this study. Cystic degeneration of the liver was reported in males in the group at the highest dose at 2 years, and there was an increase in the incidence of eosinophilic foci in both males and females at 2 years.

No significant renal changes were observed in mice. Kidney weights of rats were slightly increased at 2 years, and renal lesions (cortical tubule cell basophilia, hyaline droplets and granular and hyaline casts) were reported in male rats, mainly at the highest dietary concentration of 2500 ppm after 1 year, although an increased incidence of cortical tubule cell basophilia was also observed at 750 ppm. After 2 years, there was no further progression of these renal lesions, which were again confined to males and only at the highest dose. The NOAEL was 50 ppm, equal to 7.9 mg/kg bw per day in males and 11.5 mg/kg bw per day in females, on the basis of increased liver weights, enlarged liver, masses and nodules in the liver, and hepatocellular hypertrophy at 400 ppm, equal to 64.5 mg/kg bw per day in males and 91.9 mg/kg bw per day in females, in the 18-month dietary study in mice. Fluopicolide induced hepatocellular adenomas in male and female mice at 3200 ppm, equal to 552 mg/kg bw per day in male mice and 773 mg/kg bw per day in female mice. The NOAEL was 200 ppm, equal to 8.4 mg/kg bw per day, on the basis of increased centrilobular hypertrophy of the

liver and increased kidney weights at 750 ppm, equal to 32 mg/kg bw per day, in the 2-year dietary study of toxicity and carcinogenicity in rats. A short-term investigation of the neoplastic hepatic effects in mice given fluopicolide at a dietary concentration of 3200 ppm demonstrated increased cell proliferation after 7 days, but not after 28 days. Biochemical measurements made in these mice after 7 days demonstrated increases in hepatic cytochrome P450 content and hepatic activities of BROD, EROD and PROD enzymes, some of which were consistent with the induction of cytochrome P450 2B. A reduction in the hydroxylation of lauric acid also occurred. This pattern of changes is almost identical to the profile reported in mice treated with phenobarbital at 80 mg/kg bw per day and is indicative of a constitutive androstane receptor (CAR)–mediated response. These data are biomarkers for a proposed mode of action for fluopicolide in mouse liver that is similar to that of phenobarbital. An application of the International Programme on Chemical Safety (IPCS) conceptual framework for cancer risk assessment is described in the appendix.

The genotoxic potential of several batches of fluopicolide was investigated in a range of studies in vitro and in vivo. A small number of significant or equivocal responses were observed. A significant response with one batch in a test for mutation in bacteria was not confirmed upon repetition of the study. Another batch was associated with an equivocal response in a test for micronucleus formation in mouse bone marrow and was used in the long-term studies of toxicity and carcinogenicity in mice and rats. Current production batches are of higher purity than those used in the genotoxicity testing programme. In conclusion, the overall weight of evidence suggested that some batches of fluopicolide can have weak mutagenic properties in vitro or in vivo at toxic doses. The Meeting considered that fluopicolide at current purity levels was unlikely to present a genotoxic hazard to humans.

A significantly increased incidence of hepatocellular adenoma was observed in mice, but the Meeting proposed that these were induced by a mode of action in which CAR activation is involved. The profile of hepatotoxicity of fluopicolide, including CAR activation, is similar to that observed with phenobarbital, a chemical for which there is extensive experience of exposure in humans, but no evidence for carcinogenicity in humans. The Meeting therefore considered the liver tumours in mice to be of no relevance to humans.

On the basis of the available studies, the Meeting considered that there was no evidence of carcinogenic potential for fluopicolide administered to rats.

The Meeting concluded that fluopicolide was unlikely to be carcinogenic in humans.

In a two-generation study of fluopicolide in rats, there were increased mean absolute and relative kidney and liver weights and reduced spleen weights in males and females at 2000 ppm, the highest dose, but not at lower doses. The NOAEL for systemic toxicity in the parental generation was 500 ppm, equal to 25.5 mg/kg bw per day, on the basis of increases in liver and kidney weights of rats at 2000 ppm, equal to 103.4 mg/kg bw per day. In the multigeneration study in rats, the NOAEL for reproductive toxicity was 2000 ppm, equal to 103.4 mg/kg bw per day (the highest dose tested), for F_0 rats for the period before pairing. The overall NOAEL for pups and developing offspring was 500 ppm, equal to 25.5 mg/kg bw per day, on the basis of reduced body weight gains of pups during lactation and reduced absolute spleen and thymus weights in males and females of the F_1 and F_2 generations at 2000 ppm, equal to 103.4 mg/kg bw per day.

In a study of developmental toxicity in which rats were given fluopicolide by gavage on days 7–20 of gestation, the NOAEL for maternal toxicity and fetotoxicity was 60 mg/kg bw per day on the basis of slightly decreased body weight in dams and reduction in mean fetal body weights and crown–rump lengths in fetuses at 700 mg/kg bw per day. Further evidence of fetotoxicity at this dose was provided by increased incidences of anomalies of the thoracic vertebrae, sternebrae and ribs, as well as delayed ossification.

In a study of developmental toxicity in which rabbits were given fluopicolide by gavage on days 6–28 of gestation, the NOAEL for maternal toxicity and fetotoxicity in rabbits was 20 mg/kg

bw per day on the basis of mortality, a high incidence of premature delivery and reduction in body weight gain and food consumption in dams and reduction in fetal body weights and fetal crown–rump lengths at a dose of 60 mg/kg bw per day.

The Meeting concluded that fluopicolide causes fetotoxicity and skeletal anomalies only at doses that are also maternally toxic.

In a study of neurotoxicity in rats given a single dose of fluopicolide by gavage, the NOAEL was 100 mg/kg bw, on the basis of reduction in body temperature and increased incidence of excessive grooming in females at 2000 mg/kg bw. In a 90-day study of neurotoxicity in rats given diets containing fluopicolide, the NOAEL was 200 ppm, equal to 15.0 mg/kg bw per day, on the basis of impaired growth and histopathological changes in the liver and kidney at 1400 ppm, equal to 106.6 mg/kg bw per day. The NOAEL for neurotoxicity was 10 000 ppm, equal to 781 mg/kg bw per day, the highest dose tested.

The Meeting concluded that fluopicolide is unlikely to cause neurotoxicity in humans.

Toxicological data on metabolites

Some aspects of the toxicology of four metabolites of fluopicolide—M-01, M-02, M04 and M-05—were reported. These metabolites are also found as residues in crops. The radiolabelled phenyl metabolite, M-01, has been subjected to kinetic and metabolic studies in rats given oral doses. The highest tissue concentrations were seen in the kidney and liver of rats at 10 mg/kg bw and in the skin and fur, kidneys and liver of rats at 150 mg/kg bw. Tissue concentrations increased by approximately 5-fold for a 15-fold increase in dose, and multiple dosing did not indicate any bioaccumulation. The radiolabel was eliminated mostly in the urine (approximately 82% of the administered dose), with low levels eliminated in the faeces (approximately 13% of the administered dose). The rate of elimination was relatively slow. Biotransformation showed no sex-specific or dose-dependent differences and consisted of hydrolysis of the amide group, hydroxylation and subsequent conjugation with either glucuronic acid or sulfate, and the loss of a chlorine atom followed by glutathione conjugation and further metabolism of the glutathione group to mercapturic acid or *S*-methyl metabolites.

The pyridyl metabolite, M-02, was well absorbed, with minimum mean absorption calculated to be 87%. Elimination was rapid from both male and female rats, with at least 90% of the total administered radioactivity eliminated within the first 48 h after dosing. The total recovery in urine accounted for about 80% of the administered dose, with faecal elimination accounting for about 7% of the administered dose. Unchanged M-02 accounted for most of the eliminated material.

The acute toxicity of M-01 is relatively low, with an oral LD_{50} of 2000 mg/kg bw in male rats and 500 mg/kg bw in female rats, whereas the acute toxicity of M-02, M-04 and M-05 can be described as very low, oral LD_{50} values being >2000 mg/kg bw for M-02 and M-04 in rats and >5000 mg/kg bw for M-05. Thus, only M-01 has an acute toxicity that is higher than that of fluopicolide.

In a 28-day study of dietary toxicity with M-02 in rats, no treatment-related change was seen in mean terminal body weights, mean absolute and relative organ weights, or gross postmortem or microscopic examination. The NOAEL for M-02 was 20 000 ppm, equal to 1574 mg/kg bw per day, the highest dose tested.

In a 28-day study of dietary toxicity with M-04 in rats, the NOAEL was 2000 ppm, equal to 159.2 mg/kg bw per day, on the basis of lower body weights, reduced haemoglobin concentration, increased plasma cholesterol concentration, increased liver and kidney weights and histological findings in the liver, kidney and thyroid at 20 000 ppm, equal to 1775 and 1931 mg/kg bw per day in males and females, respectively.

In a 28-day dietary study of toxicity with M-05 in rats, the NOAEL was 2000 ppm, equal to 152 mg/kg bw per day, on the basis of clinical signs, reductions in body weight and food consumption

and renal effects at 20 000 ppm, equal to 1775 mg/kg bw per day. An increase in liver weight at this, the highest, dose was not accompanied by microscopic changes.

In a 13-week dietary study of toxicity with M-01 in rats, no effects on liver or kidney function were observed. The NOAEL for M-01 was 180 ppm, equal to 14 mg/kg bw per day, on the basis of reductions in food consumption and body weight gain and reduced skeletal muscle tone at 600 ppm, equal to 49 mg/kg bw per day.

In a 13-week study of dietary toxicity with M-01 in dogs, the NOAEL was 300 ppm, equivalent to 22.5 mg/kg bw per day, on the basis of clinical signs and increases in liver weight and serum alkaline phosphatase activity at 2000 ppm, equivalent to 150 mg/kg bw per day. Increased liver weight at 300 ppm was not considered to be toxicologically significant.

In a 2-year dietary study with M-01 in dogs, the NOAEL was 180 ppm, equal to 4.5 mg/kg bw per day, on the basis of reduced body weight gain at 500 ppm, equal to 12.5 mg/kg bw per day.

In a 2-year study with M-01 in rats, the liver was the primary target for toxicity. These effects were largely confined to females and consisted of increased incidences of vacuolation, fat deposition, hepatocyte degeneration, eosinophilic foci and basophilic foci in the liver. There was also an increased incidence of hepatocellular adenomas that was marginally statistically significant ($P = 0.05$). No relevant data on historical controls were available to assist in an evaluation of this result. The NOAEL was 60 ppm, equal to 2.0 mg/kg bw per day, based on body weight reductions, increased incidences of eosinophilic and basophilic foci in the livers and fat deposition and cellular degeneration in the liver at 100 ppm, equal to 3.5 mg/kg bw per day.

These metabolites were tested for genotoxicity in an adequate range of assays. M-01 and M-04 were tested in vitro and in vivo, whereas M-02 was tested in vitro. M-05 was tested in vitro for mutagenicity in bacteria and V79 cells. No evidence of genotoxicity was observed for M-01, M-02 or, in a more limited test profile, M-05. There was no evidence for mutagenicity with M-04 in bacteria or V79 cells, although the proportion of chromosomal aberrations was increased in treated human lymphocytes in culture. In a test for unscheduled DNA synthesis in rat liver in vivo and an assay for micronucleus formation in mouse bone marrow cells in vivo, there was no evidence for genetic toxicity or mutagenicity with M-04. The Meeting noted, for consideration of ARfDs, that clinical signs of toxicity were observed in the dose range–finding study in mice given a single dose of 100 mg/kg bw (the lowest dose tested) by gavage. Thus, the mutagenic (clastogenic) activity observed in vitro was not confirmed in vivo. The Meeting concluded that M-01, M-02 and M-04 are unlikely to be genotoxic.

In a three-generation study with M-01 in rats, the NOAEL for parental toxicity and for reproductive toxicity was 180 ppm, equal to 13.5 mg/kg bw per day (the highest dose tested), on the basis of the absence of parental toxicity and reproductive toxicity. The NOAEL for fetal toxicity was 100 ppm, equal to 7.5 mg/kg bw per day, on the basis of increased liver weights relative to body weights at 180 ppm, equal to 13.5 mg/kg bw per day.

In a study of developmental toxicity in rabbits given M-01 by gavage on days 7–19 of gestation, the NOAEL for maternal toxicity and fetotoxicity was 30 mg/kg bw per day on the basis of maternal mortality and abortions in dams and slightly reduced birth weights at 90 mg/kg bw per day. M-01 was not teratogenic in the study of developmental toxicity in rabbits. Data for individual rabbits were examined for effects that may have been produced by a single or small number of doses, but none were found.

In conclusion, in studies of acute toxicity and in long-term studies of toxicity, M-01 is more toxic than fluopicolide, whereas the data show that the metabolites M-02, M-04 and M-05 are less toxic than the parent fluopicolide. In the case of M-04, there are clear similarities with the toxicity profile of fluopicolide. A weak tumorigenic response to M-01 in the liver of female rats would appear to have no significance for an interpretation of the fluopicolide-associated liver tumours, which were

found in male and female mice, but not in rats. In view of the lack of genotoxicity and the occurrence of benign tumours only at a high dose, the Meeting concluded that M-01 was unlikely to be carcinogenic in humans at estimated dietary levels of exposure.

There have been no reported incidents of adverse reactions during the pilot-scale manufacture or formulation of fluopicolide. No further information on medical surveillance or poisoning incidents was available.

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

Toxicological evaluation

Fluopicolide and M-01 (2,6-dichlorobenzamide)

Fluopicolide

An acceptable daily intake (ADI) of 0–0.08 mg/kg bw was established for fluopicolide based on the NOAEL of 7.9 mg/kg bw per day, identified on the basis of organ weight increases and gross and microscopic changes in the liver and kidneys in an 18-month dietary study of toxicity and carcinogenicity in mice, supported by the NOAEL of 8.4 mg/kg bw per day identified on the basis of histopathological changes in the liver and increased kidney weights in a 2-year dietary study of toxicity and carcinogenicity in rats, and with a safety factor of 100.

An ARfD of 0.6 mg/kg bw was established for women of child-bearing age based on a NOAEL of 60 mg/kg bw per day identified on the basis of a marginally increased incidence of skeletal defects of the vertebrae and sternebrae, which might be attributable to a single exposure to fluopicolide, at 700 mg/kg bw per day in a study of developmental toxicity in rats, and with a safety factor of 100.

The Meeting concluded that the establishment of an ARfD for the general population was not necessary for fluopicolide on the basis of its low acute toxicity, the lack of evidence for any acute neurotoxicity and the absence of any other toxicologically relevant effect that might be attributable to a single dose.

M-01 (2,6-dichlorobenzamide)

An ADI of 0–0.02 mg/kg bw was established for the fluopicolide metabolite M-01 based on the NOAEL of 2.0 mg/kg bw per day identified on the basis of microscopic changes in the liver in a 2-year dietary study of toxicity and carcinogenicity in rats, supported by the NOAEL of 4.5 mg/kg bw per day identified on the basis of reduced body weight gain in a 2-year dietary study of toxicity in dogs, and with a safety factor of 100.

The Meeting concluded that the establishment of an ARfD for the general population should be considered based on the finding of mortality at single oral doses of less than 500 mg/kg bw in female rats. A lowest-observed-adverse-effect level (LOAEL) of 100 mg/kg bw was identified on the basis of clinical signs of toxicity in a dose range–finding study in mice given a single dose of M-01, but this study did not provide sufficient detail for it to be used as the basis for an ARfD by itself. In the absence of adequate data, an ARfD for the general population was established for the metabolite based on the value of 0.6 mg/kg bw for the parent compound. This value is derived from a study of developmental toxicity in rats and a safety factor of 100, as described above. The ARfD derived from a study with fluopicolide is sufficiently protective for application to the metabolite M-01, owing to the large dose spacing between the LOAEL and the NOAEL.

Levels relevant to risk assessment for fluopicolide

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity	Toxicity	50 ppm, equal to 7.9 mg/kg bw per day	400 ppm, equal to 64.5 mg/kg bw per day
		Carcinogenicity	400 ppm, equal to 64.5 mg/kg bw per day	3200 ppm, equal to 552 mg/kg bw per day
Rat	Two-year studies of toxicity and carcinogenicity	Toxicity	200 ppm, equal to 8.4 mg/kg bw per day	750 ppm, equal to 32 mg/kg bw per day
		Carcinogenicity	2500 ppm, equal to 109.4 mg/kg bw per day[a]	—
	Two-generation study of reproductive toxicity	Reproductive toxicity	2000 ppm, equal to 103.4 mg/kg bw per day[a]	—
		Parental toxicity	500 ppm, equal to 25.5 mg/kg bw per day	2000 ppm, equal to 103.4 mg/kg bw per day
		Offspring toxicity	500 ppm, equal to 25.5 mg/kg bw per day	2000 ppm, equal to 103.4 mg/kg bw per day
	Developmental toxicity study	Maternal toxicity	60 mg/kg bw per day	700 mg/kg bw per day
		Embryo and fetal toxicity	60 mg/kg bw per day	700 mg/kg bw per day
Rabbit	Developmental toxicity study	Maternal toxicity	20 mg/kg bw per day	60 mg/kg bw per day
		Embryo and fetal toxicity	20 mg/kg bw per day	60 mg/kg bw per day
Dog	Three-month study of toxicity	Toxicity	70 mg/kg bw per day	1000 mg/kg bw per day

[a] Highest dose tested.

Levels relevant to risk assessment for M-01 (2,6-dichlorobenzamide)

Species	Study	Effect	NOAEL	LOAEL
Mouse	Dose range–finding study of toxicity for a test of micronucleus formation	Toxicity	—	100 mg/kg bw
Rat	Two-year studies of toxicity and carcinogenicity	Toxicity	60 ppm, equal to 2.0 mg/kg bw per day	100 ppm, equal to 3.5 mg/kg bw per day
		Carcinogenicity	180 ppm, equal to 5.7 mg/kg bw per day	500 ppm, equal to 17.6 mg/kg bw per day
	Two-generation study of reproductive toxicity	Reproductive toxicity	180 ppm, equal to 13.5 mg/kg bw per day[a]	—
		Parental toxicity	180 ppm, equal to 13.5 mg/kg bw per day[a]	—
		Offspring toxicity	100 ppm, equal to 7.5 mg/kg bw per day	180 ppm, equal to 13.5 mg/kg bw per day
Rabbit	Developmental toxicity study	Maternal toxicity	30 mg/kg bw per day	90 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day	90 mg/kg bw per day
Dog	Two-year study of toxicity	Toxicity	180 ppm, equal to 4.5 mg/kg bw per day	500 ppm, equal to 12.5 mg/kg bw per day

[a] Highest dose tested.

Estimate of acceptable daily intakes for humans

Fluopicolide	0–0.08 mg/kg bw
M-01[1]	0–0.02 mg/kg bw

Estimates of acute reference doses

Fluopicolide	0.6 mg/kg bw for women of child-bearing age
	Unnecessary for the general population
M-01	0.6 mg/kg bw

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

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

Critical end-points for setting guidance values for exposure to fluopicolide and its metabolite M-01 (2,6-dichlorobenzamide)

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Moderately rapid and moderately extensive, at least 80%
Distribution	Distributed throughout the body; higher concentrations in liver, kidney and blood
Potential for accumulation	No evidence for accumulation
Rate and extent of excretion	Moderately rapid, >70% within 24 h, but subsequently low rate, with 95% within 48 h, mainly in faeces
Metabolism in animals	Extensively metabolized; biotransformations observed included aromatic ring hydroxylation, hydrolysis, dealkylation, acetylation, oxidative *N*-dealkylation and conjugation with glucuronic acid, sulfate and glutathione. Up to 46 radiolabelled components in urine and faeces
Toxicologically significant compounds (animals, plants and environment)	Parent, M-01

Acute toxicity	Fluopicolide	2,6-Dichlorobenzamide (M-01)
Rat, LD_{50}, oral	>5000 mg/kg bw	2000 mg/kg bw in males, 500 mg/kg bw in females
Rat, LC_{50}, inhalation	>5.2 mg/l[a] (4 h)	No data
Rat, LD_{50}, dermal	>5000 mg/kg bw[a]	No data
Rabbit, dermal irritation	Not an irritant	No data
Rabbit, ocular irritation	Slightly, transiently irritating	No data
Guinea-pig, dermal sensitization	Not sensitizing (Magnusson and Kligman test)	No data
Short-term studies of toxicity		
Target/critical effect	Liver, kidney	Body weight gain; muscle tone
Lowest relevant oral NOAEL	7.4 mg/kg bw per day (3-month study in rats)	14 mg/kg bw per day (3-month study in rats
	300 mg/kg bw per day (1-year study in dogs)	4.5 mg/kg bw per day (2-year study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day[a] (28-day study in rats)	No data

[1] 2,6-Dichlorobenzamide.

Genotoxicity	Fluopicolide	2,6-Dichlorobenzamide (M-01)
	A small number of inconsistent positive or equivocal responses were observed, but the overall weight of evidence is that it is unlikely to be genotoxic.	Not genotoxic
Long-term studies of toxicity and carcinogenicity		
Target/critical effect	Liver	Liver
Lowest relevant NOAEL	7.9 mg/kg bw per day (18 month study in mice)	60 ppm, equal to 2 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Benign liver tumours in mice that are of no human relevance, based on mode of action	Benign liver tumours in rats that are unlikely to pose a risk to humans
Reproductive toxicity		
Reproductive target/critical effect	No reproductive toxicity	No reproductive toxicity
Lowest relevant reproductive NOAEL	2000 ppm, equal to 103 mg/kg bw per day	180 ppm, equal to 13.5 mg/kg bw per day
Developmental target/critical effect	Not teratogenic; abortions, total litter loss, reduced fetal body weight and pup body weight during lactation, delayed ossifications, vertebral and sternebral defects	Not teratogenic; abortions, reduced fetal body weight, increased relative liver to body weight
Lowest relevant developmental NOAEL	60 mg/kg bw per day (rat)	30 mg/kg bw per day (rabbit)
	20 mg/kg bw per day (rabbit)	
Neurotoxicity/delayed neurotoxicity		
	No signs of neurotoxicity	No data
Other toxicological studies		
	Induction of liver xenobiotic metabolizing enzymes in female mice and male and female rats	No data
	Several metabolites that are also crop residues have been investigated, but only M-01 (2,6-dichlorobenzamide) was more toxic than fluopicolide in a single-dose and a long-term study.	
Medical data		
	No reports of toxicity in workers exposed during pilot-scale manufacture or formulation	

[a] Highest dose tested.

Summary

	Value	Study	Safety factor
Fluopicolide			
ADI	0–0.08 mg/kg bw	Mouse, 18-month study of toxicity and carcinogenicity	100
ARfD	0.6 mg/kg bw for women of child-bearing age	Rat, study of developmental toxicity	100

Summary

	Value	Study	Safety factor
M-01 (2,6-dichlorobenzamide)			
ADI	0–0.02 mg/kg bw	Rat, 2-year study of toxicity and carcinogenicity	100
ARfD	0.6 mg/kg bw general population	Rat, study of developmental toxicity on the parent compound	100

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Appendix 1: Application of the IPCS conceptual framework for cancer risk assessment

A1. Hepatocarcinogenesis in mice

A1.1 Introduction

All information relating to a mode of action (MOA) has been summarized in this document, so, rather than redescribe the data in this section, reference will be made to the relevant paragraphs. The analytical approach applied to the postulated MOA is based on the methods developed by the IPCS (Sonich-Mullin et al., 2001; Boobis et al., 2006); however, justification for the exercise of this framework rests on an affirmative response to the question, "Is there a case to answer?" or, in other words, "Is it clear that the compound induced an increase in the incidence of any particular type of tumour?"

The answer to this question must come from an evaluation of the evidence for carcinogenicity in the rodent models. Statistically significant increases in the incidences of hepatocellular adenomas in mice were observed at the highest dose level (3200 ppm) in both males (11/50) and females (16/50). In neither sex was there any indication of a response at the next lower dose level (400 ppm), and there were no increases in hepatocellular carcinomas or any other liver tumour in mice at any dose level. In rats, there were no treatment-related tumour findings in any organ, although non-neoplastic changes were observed in the liver, about which more will be said below.

A1.2 Postulated mode of action (theory of the case)

Fluopicolide induces liver enzymes, including cytochrome P450 (CYP) isozymes, by activation of nuclear receptors; this is followed by the induction of liver cell proliferation and hypertrophy. With continued exposure, the increase in liver growth continues, and foci of altered hepatocytes (FAH) appear. Such foci ultimately progress to neoplasia (Bannasch, Haertel & Su, 2003).

A1.3 Key events

The key events in the fluopicolide MOA for liver tumour formation in mice include the following:

1) Activation of nuclear receptors is most probably an early step, although no data have been presented that demonstrate an interaction of fluopicolide with any nuclear receptors. Nevertheless, it is likely that this does occur, given that enzyme induction results from short-term administration of the compound to mice.

2) Induction of CYP enzymes. Dietary administration of fluopicolide at 3200 ppm to female mice for 7 days resulted in increased activities of BROD, EROD and PROD and a reduction in lauric acid hydroxylation in a pattern that was almost identical to that produced when phenobarbital was administered to female mice of the same strain at 80 mg/kg bw per day for 7 days (see Table 30) and is indicative of a constitutive androstane receptor (CAR)–mediated response. This contrasts with the different pattern of enzyme responses obtained when the peroxisome proliferator, clofibrate, was administered at 300 mg/kg bw per day (Table 30).

3) Induction of cell proliferation, which is transient, in the liver. Dietary administration of fluopicolide at 3200 ppm to female mice for 7 or 28 days and the simultaneous drinking-water administration of BrdU for 7 days resulted in significantly increased BrdU labelling indices in the livers at 7 days, but not at 28 days (Table 29).

4) Hypertrophy and hyperplasia develop in the enlarging liver. There were dose-related increases in the incidence and severity of hepatocellular, centrilobular, diffuse hypertrophy at 3 months in male and female mice given diets of 800 or 3200 ppm (Table 7). This trend continued at 12 months and 18 months (Table 17) and was reflected in the dose-related increases in liver weights in the 400 and 3200 ppm dose groups in comparison with the controls at 1, 3, 12 and 18 months.

5) Liver weight increases are induced by treatment. There were increases in absolute and relative liver weights of 48% and 56%, respectively, within 7 days in female mice administered fluopicolide at 3200 ppm. Similar liver weight increases were observed in mice after dietary fluopicolide administration for 1 month (3200 ppm), 3 months (800 ppm), 12 months (400 ppm) and 18 months (400 ppm).

6) FAH develop. The incidence of FAH at 18 months was clearly significantly increased in male and female mice of the 3200 ppm dose group (Table 17). Although the incidences were also greater in the 50 and 400 ppm dose groups than in the control group, there was no dose relationship over

these dose levels. There has, however, been no description of size progression or multiplicity of the FAH in mouse liver.

7) Hepatocellular adenomas develop from within FAH. Whether or not there was a development of adenomas from FAH has not been ascertained. It may be commented that such a demonstration is always likely to be difficult and would probably require either a complete re-evaluation of the liver sections from existing archives or a new experiment examining the temporal sequencing of lesion development at frequent intervals late in the carcinogenicity study. The incidences of hepatocellular adenomas in mice (out of group sizes of 50) of the 0, 50, 400 and 3200 ppm dose groups, respectively, were 5, 0, 5 and 11 for males and 1, 2, 0 and 16 for females. The more frequent occurrence of tumours in females of the 3200 ppm group in comparison with the controls justifies further investigations targeting female mice in preference to male mice.

A1.4 Concordance of dose–response relationships

All of the features considered to contribute to the key events occurred in dose–response relationships, as described above. In addition, it is clear 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. Thus, adenomas and FAH were increased only at 3200 ppm, whereas hepatocellular hypertrophy occurred at 400 and 3200 ppm (not at 50 ppm) at 18 months, at 800 and 3200 ppm (not at 200 ppm) at 3 months and at 3200 ppm in the 1-month mechanistic study, where there was but a single dose level. Enzyme induction was measured only at 7 days, and cell proliferation was measured at 7 days and 1 month, both at a single dose level of 3200 ppm. Thus, within the limitations of the study designs, there was a completely logical concordance of dose–response relationships.

A1.5 Temporal association

Again, within the limitations of study design, the temporal relationships followed a logical pattern. Enzyme induction and cell proliferation in the liver were observed after just 7 days (although cell proliferation had returned to control levels within 1 month), and liver weight increases were observed in this experiment, whereas microscopically recognized cell hypertrophy was first observed at 1 month and 640 ppm in CD-1 mice and at 3 months and 800 ppm in C57BL/6JICO mice. FAH and adenomas first appeared at 12 months and increased in incidence by 18 months.

A1.6 Strength, consistency and specificity of association of tumour response with key events

Liver weight increases, hepatocellular hypertrophy and the occurrence of FAH were all expected and were all observed to occur in male mice as well as female mice. Experiments specifically designed to investigate some aspects of the MOA of fluopicolide (liver enzyme induction and cell proliferation) were conducted in female mice. These demonstrated increased cell proliferation, as well as a profile of CYP enzyme induction that was consistent with CAR activation. Further data that might provide evidence for a consistency in the response could come from male mice, in which there was also an increase in hepatocellular adenoma incidence. Unfortunately, no studies of liver enzyme induction or of cell proliferation were undertaken on male mice. While this difference in experimentation might mask a potential for a lack of consistency, other key events were observed in male mice in other experiments that were consistent with an association with tumour response.

The specificity of these events is open to verification if similar studies are made on rats, in which dietary fluopicolide administration was not associated with an increase in liver tumours. In

a 7-day dietary study in which fluopicolide was administered at 2500 ppm to male and female rats, total CYP was increased in males, but not in females. Although BROD, EROD and PROD activities were increased in both males and females, the increases in BROD and PROD activities were much greater in male rats than in females. Lauric acid hydroxylation was slightly reduced in both male and female rats (Table 31). The changes in female mice, in which tumours were observed, and male rats, in which tumours were not observed, were similar. The female rat response tended to be less like those of the male rat and female mouse. It might also be added that in a parallel study of phenobarbital, the pattern of enzyme induction in male rats was also similar to the pattern of phenobarbital-induced enzymes in female mice. There were, however, quantitative differences in female rats. The level of PROD induction was 13-fold following phenobarbital treatment, but only 2.3-fold following fluopicolide administration. Similarly, the total CYP level was increased 1.8-fold by phenobarbital, but hardly at all (1.1-fold) after fluopicolide. These differences may not be due simply to suboptimal dosing with fluopicolide, as PROD induction was 12-fold and 8.8-fold in male rats dosed with phenobarbital and fluopicolide, respectively.

Liver weights in rats were increased by dietary fluopicolide at 20 000 ppm after 1 month and 3 months and at 2500 ppm after 24 months. In fluopicolide-treated rats, centrilobular hepatocytic hypertrophy was increased in incidence and severity in males and females given diets containing 200, 2000 or 20 000 ppm fluopicolide after 1 month (Table 9). There was also increased incidence and severity of hypertrophy after 3 months of treatment with fluopicolide at 1400 or 20 000 ppm for males, but only after 20 000 ppm for females (Table 11). This tendency towards a higher sensitivity among male rats was also observed at 12 months, in which 750 or 2500 ppm fluopicolide produced centrilobular hypertrophy incidences of 14 out of 20 and 19 out of 20, respectively, in male rats (0/20 in controls), but not at all in female rats (Table 22). This sex difference was observed again at 24 months, when the incidences of centrilobular hypertrophy in groups of 60 at the 0, 50, 200, 750 and 2500 ppm dose levels, respectively, were 0, 0, 2, 9 and 18 in males and 0 in all groups of females (Table 22). No fluopicolide treatment–related tumour findings were observed in the liver of either male or female rats.

Phenobarbital is often considered to be carcinogenic in rats as well as mice, but there are, in fact, few studies that might demonstrate such an effect. Only three studies are mentioned in the International Agency for Research on Cancer (IARC, 2001) review: one used male and female Wistar rats in which most of the modest number of liver tumours were found between weeks 100 and 130 (cumulative incidences being 13/36 in males and 9/29 in females) (Rossi et al., 1977); the remaining two studies tested male Fischer 344 rats only. In one of these (Butler, 1978), there were small foci of nodular hyperplasia in 11 out of 33 treated rats that lived 80 weeks, whereas none developed in the controls. Ward (1983) gave a group of 30 male Fischer 344/NCr rats (874 days of age on average) drinking-water containing sodium phenobarbital at a concentration of 500 mg/l for up to 233 days. There was no significant difference in the number of rats with hepatocellular adenomas between the control (10/30) and the treated groups (14/30), although the total numbers (multiplicity) of hepatocellular adenomas were greater in the treated than in the control group (sodium phenobarbital: 5 basophilic, 64 eosinophilic; control: 14 basophilic, 2 eosinophilic). Thus, the difference in carcinogenic response between rats and mice treated with fluopicolide may, in fact, form another similarity with phenobarbital, although the lack of a difference, at least in males, insofar as the preneoplastic key events are concerned is an interesting point that does not appear to have been addressed.

A1.7 Biological plausibility and coherence

The MOA proposed for the induction of liver tumours in female mice by fluopicolide is plausible and cohesive, as the data show a substantial similarity to the MOA that has been proposed (and which appears to be widely accepted) for phenobarbital (Whysner et al., 1998). Liver tumour induction by

phenobarbital is characterized by increased liver weight, hepatocellular hypertrophy (particularly of the centrilobular region) and hepatocellular hyperplasia, as identified by using techniques such as BrdU labelling. Phenobarbital hepatocarcinogenicity is also characterized by the appearance of FAH and a higher sensitivity of mice than rats to the development of liver neoplasia.

A1.8 Other modes of action

Liver tumours are common in long-term studies in rats and mice, and a number of potential MOAs other than enzyme induction have been suggested that may lead to the development of these tumours. These include 1) genetic activity, including DNA reactivity, 2) cytotoxicity and proliferative regeneration, 3) estrogenic stimulation and 4) alternative receptor-mediated mechanisms.

The genotoxic potential of several batches of fluopicolide was investigated in a range of in vitro and in vivo studies, in which a small number of significant or equivocal responses were observed. One batch was associated with an equivocal response in a mouse bone marrow micronucleus induction test and was used in the chronic toxicity and carcinogenicity tests with mice and rats. However, this equivocal response is unlikely to be a demonstration of sufficient activity to cause tumours (Table 24).

There was no indication of hepatotoxic effects, such as peroxisome proliferation or chronic degeneration, in the general toxicity studies performed on fluopicolide that might suggest cycles of degeneration and regenerative hyperplasia. 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. 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).

A1.9 Uncertainties, inconsistencies and data gaps

The weight of the evidence suggests that the MOA for fluopicolide-induced liver tumours in female mice is based on CYP enzyme induction in the liver. It is being assumed that the increase in hepatocellular adenomas in male mice results from a similar MOA, but it should be clearly understood that no evidence for enzyme induction by fluopicolide in male mice has been presented in this document.

The close similarity in the pattern of CYP enzyme induction by fluopicolide in female mice and male rats poses a difficulty in the ready acceptance of the proposed MOA, as no liver tumours were observed in rats. On the other hand, these enzyme patterns are similar to those produced by phenobarbital in female mice and male rats, species that also show differences in susceptibility to phenobarbital tumorigenesis. It is at least to be considered, therefore, that the enzyme induction patterns observed with fluopicolide and phenobarbital are only indications of some other receptor-mediated pathway leading towards tumorigenesis that have yet to be explored.

It has been emphasized that the quantitative assessment of morphometric changes in FAH are essential in the evaluation of hepatic neoplasia (Bannasch, Haertel & Su, 2003); however, no such assessment has been undertaken in the case of fluopicolide. This omission could be important when making any extrapolation of the findings to humans.

Another uncertainty rests in the observations of hypertrophy and hyperplasia, which are listed as key events. As these also occur in rats, which are resistant to the hepatocarcinogenicity of both phenobarbital and, at the doses tested, fluopicolide, at least in a standard 2-year study, it can be concluded that hypertrophy and hyperplasia may be essential but are not sufficient to the pathway leading to hepatocarcinogenesis.

A1.10Assessment of postulated mode of action

A case has been proposed to explain the induction of hepatocellular adenomas in female mice exposed to fluopicolide. Some weaknesses have been described above, among them being an absence of a companion data set that might be applied to male mice. Also, the sharing of some key events with rats—for which no liver tumours were induced by fluopicolide; even for phenobarbital (with which fluopicolide has been compared), the appearance of excess hepatocellular tumours was very late—detracts from their significance in the course of events. Is it possible that, should the rat carcinogenicity study with fluopicolide have been prolonged for some more weeks, liver tumours would have emerged?

Human relevance analysis

1. Is the weight of evidence sufficient to establish a mode of action in animals?

There are many toxic effects of fluopicolide that are consistent with an MOA based on receptor-mediated gene activation and that have similarities with properties of phenobarbital, and it has been concluded that the weight of evidence supports the MOA proposed for mouse hepatotumorigenesis. Weaknesses have also been recognized, which, in turn, render difficult any analysis of the human relevance of the proposed MOA for liver tumours in mice.

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 nuclear receptor involved in phenobarbital-induced liver enzyme induction is mainly CAR. Although CAR is also expressed in human hepatocytes, CYP induction in human liver may be mediated more by pregnane X receptor (PXR) than by CAR (Moore et al., 2003). Furthermore, recent research has strongly suggested that human CAR and PXR introduced to mouse knockout models lacking the mouse genes for these functions only result in hepatic hypertrophy and not hepatic hyperplasia when these modified mice are treated with either phenobarbital or chlordane (Ross et al., 2010). The conclusion that humans would not be expected to respond in the same way as mice applies more to phenobarbital than to fluopicolide, as the involvement of CAR or any other nuclear receptor in fluopicolide-induced liver enzyme induction, although likely, has not been demonstrated.

The detection of phenotypically similar FAH in various animal models and in humans prone to the development of, or actually bearing, hepatocellular carcinomas favours the extrapolation from data obtained in rodents to humans; hence, the human relevance cannot be reasonably excluded. The majority of the early preneoplastic FAH are smaller than a liver lobule, which has an average diameter of 1–2 mm in both rodents and humans. This small size precludes a non-invasive identification by all imaging procedures currently available. The reported frequent finding of FAH in fine-needle biopsies of patients suffering from chronic liver diseases opens new perspectives for secondary prevention of human hepatocellular carcinoma (Su et al., 1997; Su & Bannasch, 2002), but it also reduces the confidence with which it can be asserted that the rodent-based MOA can be dismissed as having any human relevance.

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?

The LOAEL and the NOAEL for liver tumours in mice treated continuously with dietary fluopicolide for 18 months are 552 mg/kg bw per day and 64.5 mg/kg bw per day, respectively. These values can be compared with the ADI that was established of 0–0.08 mg/kg bw on the basis of tox-

icity in mice with a NOAEL of 7.9 mg/kg bw per day and a 100-fold safety factor. The NOAEL is therefore approximately 800-fold higher than the ADI, and the LOAEL for liver tumours in mice is approximately 7000-fold higher than the ADI. These values of 800-fold and 7000-fold are expressions of a very large margin of exposure (MOE), which is the ratio between a reference point on the dose–response data from experimental or epidemiological studies and the estimated human exposure via residues.

Because there is some uncertainty regarding the mode of action of fluopicolide in mice (CAR involvement reasonably anticipated, but not having been demonstrated), consideration of quantitative differences in key events between mice and humans is not considered to be a sound procedure. Thus, the data that are most relevant for quantitative comparison are those outlined in the preceding paragraph. The incidences of FAH are also significantly increased only at the dose level at which tumour incidence is increased, although hypertrophy was observed at lower dose levels. These latter changes are quite likely adaptive and not indicative of any obligatory adverse outcome.

4. Statement of confidence, analysis and implications

The likelihood of CAR involvement in the generation of liver tumours in mice treated with fluopicolide is reasonable, but certain key experiments that might strengthen the argument are lacking. Should CAR be involved in mouse carcinogenesis, then there is evidence that humans are unlikely to respond similarly, because the consequence of CAR involvement in humans is hypertrophy rather than hyperplasia. Full confidence cannot be attributed to this approach; therefore, consideration has also been given to an MOE approach. In the context of carcinogens with no threshold doses that are believed to act through a genotoxic MOA, MOE values of 10 000–25 000 for estimated human exposure via residues have been proposed by FAO/WHO (2005), and MOE values in excess of 10 000 have been proposed by EFSA (2005) (discussed in Barlow et al., 2006). These are clearly much greater ratios than the 700 that is applicable to fluopicolide. However, the genotoxicity database for fluopicolide does not support genotoxicity being a likely MOA accounting for its hepatocarcinogenicity in mice, and therefore lower values for the MOE can be considered.

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METAFLUMIZONE

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Explanation

Metaflumizone is the International Organization for Standardization (ISO)–approved common name for (*EZ*)-2′-[2-(4-cyanophenyl)-1-(α,α,α-trifluoro-*m*-tolyl)ethylidene]-4-(trifluoromethoxy)-

carbanilohydrazide (Chemical Abstracts Service [CAS] No. 139968-49-3), which is a mixture of *E* and *Z* isomers (ratio, approximately 9:1). Metaflumizone is a novel insecticide of the semicarbazone class, which acts by blocking voltage-dependent sodium channels of the nervous system, causing paralysis of the insect. Metaflumizone was evaluated at the request of the Codex Committee on Pesticide Residues and was not evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR).

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.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The absorption, distribution, metabolism and elimination (ADME) of ^{14}C-labelled metaflumizone in male and female Sprague-Dawley (Crl:CD BR and Crl:CD IGS BR) rats were investigated at dose levels of 30 and 1000 mg/kg body weight (bw) (Afzal & Zulalian, 2002). The experiments were performed with metaflumizone labelled either in the benzonitrile ring (B-label) or in the trifluoromethoxyphenyl ring (T-label). To facilitate structure elucidation, ^{13}C/^{15}N isotope labels were added to the ^{14}C-labelled substances. The study design is summarized in Tables 1 and 2.

A pilot study was conducted to determine whether collection of volatiles would be necessary. In the expired air of the animals dosed with B- or T-labelled [^{14}C]metaflumizone, no radioactivity was found. Therefore, all other experiments were carried out without collection traps for the volatiles.

Pharmacokinetics study: Five groups were treated orally with either an aqueous 0.5% carboxymethyl cellulose (CMC) dosing vehicle or B- or T-labelled metaflumizone in 0.5% aqueous CMC. Each treatment group consisted of four males and four females, with the vehicle control groups being composed of one male and one female rat. Blood samples were collected from the control group and the B-labelled groups at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144 and 168 h post-dose and from the T-labelled groups at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 72, 120, 168, 216, 264 and 336 h post-dose. The pharmacokinetic parameters were calculated using an extravascular input non-compartmental model by a validated WinNonlin software on the total radioactivity concentration–time blood curves.

Blood radioactivity in rats dosed with B-labelled metaflumizone at 30 mg/kg bw reached maximal concentrations in 10 h and 12 h for males and females, respectively. The elimination half-life ranged from 44 to 48 h. However, following a single oral dose of the T-labelled metaflumizone, a higher maximum blood concentration was reached in 15 h for the male rat and 23 h for the female rat. The elimination half-life of total radioactivity was 3–7 times longer for the T-label than for the B-label, ranging from 139 h for the male rat to 325 h for the female rat. The areas under the concentration–time blood curve ($AUC_{0-\square}$) for the rats treated with T-label were 8–11 times larger than those found in the rats treated with B-label (Table 3).

Table 1. Study design for ADME studies with metaflumizone

Study section	Test group	^{14}C label	No. of males / no. of females	Nominal dose (mg/kg bw)	Sacrifice time post-dose (h)
Pharmacokinetics (single oral dose)	K-Lo-Control	NA	1/1	NA	168
	K-Lo-B	B-label	4/4	30	168
	K-Lo-T	T-label	4/4	30	336
	K-Hi-B	B-label	4/4	1000	168
	K-Hi-T	T-label	4/4	1000	336
Biliary excretion (single oral dose)	B-Lo-Control	NA	1/1	NA	72
	B-Lo-B	B-label	4/4	30	72
	B-Lo-T	T-label	4/4	30	72
	B-Hi-B	B-label	4/4	1000	72
	B-Hi-T	T-label	4/4	1000	72
Mass balance (single oral dose)	M-Lo-Control	NA	1/1	NA	168
	M-Lo-B	B-label	5/5	30	168
	M-Lo-T	T-label	5/5	30	168
	M-Hi-Control	NA	1/1	NA	168
	M-Hi-B	B-label	5/5	1000	168
	M-Hi-T	T-label	5/5	1000	168
Accumulation (14 × ^{14}C oral doses)	A-Mu-Control	NA	1/1	NA	10[a]
	A-Mu-B1	B-label	3/3	30	10[a]
	A-Mu-B2	B-label	3/3	30	48[a]
	A-Mu-B3	B-label	3/3	30	168[a]
	A-Mu-T1	T-label	3/3	30	12[a]
	A-Mu-T2	T-label	3/3	30	168[a]
	A-Mu-T3	T-label	3/3	30	288[a]
Tissue and organ distribution (single oral dose)	T-Lo-Control	NA	1/1	NA	48
	T-Lo-B1	B-label	3/3	30	2
	T-Lo-B2	B-label	3/3	30	10
	T-Lo-B3	B-label	3/3	30	48
	T-Lo-T1	T-label	3/3	30	2 (M), 1 (F)
	T-Lo-T2	T-label	3/3	30	12
	T-Lo-T3	T-label	3/3	30	288
	T-Hi-Control	NA	1/1	NA	288
	T-Hi-B1	B-label	3/3	1000	0.5 (M), 1 (F)
	T-Hi-B2	B-label	3/3	1000	8 (M), 4 (F)
	T-Hi-B3	B-label	3/3	1000	36
	T-Hi-T1	T-label	3/3	1000	2 (M), 1 (F)
	T-Hi-T2	T-label	3/3	1000	48 (M), 12 (F)
	T-Hi-T3	T-label	3/3	1000	288

From Afzal & Zulalian (2002)
F, female; M, male; NA, not applicable
[a] Hours after the 14th dose.

Table 2. Test substance characteristics in ADME studies performed with metaflumizone

Identification and characterization	[Benzonitrile ring U-^{14}C + tolunitrile-benzyl-^{13}C]metaflumizone			[Trifluoromethoxyphenyl ring-U-^{14}C + trifluoro-methoxyphenylamine-^{15}N] metaflumizone		[Trifluoromethoxy-phenyl ring-U-^{14}C + trifluoromethoxy-phenyl ring-C_1-^{13}C] metaflumizone
Test group	B-Lo-B, K-Lo-B, M-Lo-B, T-Lo-B	K-Hi-B, M-Hi-B, T-Hi-B	A-Mu-B	K-Lo-T, M-Lo-T, T-Lo-T	K-Hi-T, M-Hi-T, T-Hi-T	A-Mu-T
Lot No.	AC 12432-36	AC 12694-6	AC 12694-90	AC 12432-42	AC 12694-8	AC 12694-91
Chemical purity (%)	99.76	98.27	100.1	100	97.92	97.1
Radiochemical purity (%)	97.98	97.74	96.6	98.42	97.68	97.6
Specific radioactivity (µCi/mg)[a]	13.73	0.98	8.0	12.72	0.98	8.0
^{13}C or ^{15}N enrichment (%)	~43	NA	~50	~25	NA	~50

From Afzal & Zulalian (2002)
NA = not applicable
[a] 1 µCi = 37 kBq.

Table 3. Summary of pharmacokinetic parameters in rats after a single oral dose of metaflumizone

Parameter	Dose level (mg/kg bw)							
	30				1000			
	B-label		T-label		B-label		T-label	
	M	F	M	F	M	F	M	F
T_{max} (h)	10	12	15	23	32	27	48	23
C_{max} (ng/ml)	146	183	30	224	1 667	2 178	3 950	6 426
$t_{½}$ elim. (h)	44	48	139	325	38	42	230	402
AUC_{0-t} (h × ng/ml)	7 975	8 070	37 452	31 852	78 357	73 288	901 709	1 215 964
$AUC_{0-\infty}$ (h × ng/ml)	8 524	9 028	66 079	102 236	82 302	76 535	1 461 584	2 554 699
$Vz_{observed}$/F (ml)	47 114	43 704	20 092	27 679	129 741	155 561	53 164	55 411
$CL_{observed}$/F (ml/h)	768	748	100	62	2 503	2 624	166	90
MRT (h)	68	75	202	464	61	62	341	583

From Afzal & Zulalian (2002)
AUC_{0-t}, area under the curve from time 0 to time *t*; $AUC_{0-\infty}$, area under the curve from time 0 to infinity; CL/F, clearance (where F is fraction of dose); C_{max}, maximal concentration; F, female; M, male; MRT, maximum residence time; $t_{½}$ elim., elimination half-life; Vz/F, volume of distribution

Following a single oral dose of B-labelled metaflumizone at 1000 mg/kg bw, the results demonstrated comparable total radioactivity concentration–time blood curves for the male and female rats. Blood radioactivity reached a maximal concentration in 32 h and 27 h for males and females, respectively. The elimination half-life ranged from 38 to 42 h. However, following a single oral dose of the T-label, a higher maximum blood concentration was reached in 48 h for the male rat and 23 h for the female rat. The elimination half-life was 6–10 times longer for the T-label than for the B-

Table 4. Recovery of radiolabel in excreta and tissues of bile duct–cannulated rats at 72 h after a single oral dose of metaflumizone

Excreta/tissue	Recovery of radiolabel (% of administered dose)							
	Dose level (mg/kg bw)							
	30				1000			
	B-label		T-label		B-label		T-label	
	M	F	M	F	M	F	M	F
Urine + cage rinse	0.3	0.5	0.5	0.3	0.2	0.4	0.3	0.3
Bile	4.7	3.7	1.2	0.9	1.3	0.7	0.3	0.2
Gastrointestinal tract	0	0.1	0	0	0	0	0	0
Carcass	2.3	2.4	1.0	1.7	0.4	0.3	0.3	0.3
Total	7.2	6.6	2.7	2.9	1.9	1.4	0.8	0.7

From Afzal & Zulalian (2002)
F, female; M, male

label, ranging from 230 h for the male rat to 402 h for the female rat. The $AUC_{0-\infty}$ values for the rats treated with T-labelled metaflumizone were 18–33 times larger than those found in the rats treated with B-labelled metaflumizone.

The shorter blood clearance time as indicated by elimination half-lives and $AUC_{0-\infty}$ for the T-label suggests that T-label-derived metabolites associate more to the blood components than do B-label-derived residues.

Biliary excretion study: Groups of bile duct–cannulated rats were treated orally with an aqueous 0.5% CMC dosing vehicle or B- or T-labelled metaflumizone in 0.5% aqueous CMC at 30 or 1000 mg/kg bw. Each treatment group consisted of four males and four females, with the vehicle control groups being composed of one male and one female rat. The bile, urine and faecal samples were collected at 0–12, 12–24, 24–48 and 48–72 h post-dose. At sacrifice, cage rinse, gastrointestinal tract without contents (tissue) and residual carcass were taken and analysed. The total amount of absorption of the orally administered dose was calculated by summation of the amount of radioactivity found in the urine, gastrointestinal tract (without contents) and carcass. The absorption over a 72 h period ranged from 2.7% to 7.2% for the low dose and from 0.7% to 1.9% for the high dose (Table 4). The majority of the low and high doses (>90%) were excreted in the faeces. Biliary excretion accounted for less than 5% (0.9–4.7%) of the oral low dose and less than 2% (0.2–1.3%) of the oral high dose. Urinary excretion was also very low, at or less than 0.5% of the oral low and high doses.

Mass balance study: Groups of rats were treated orally with a 0.5% aqueous CMC dosing vehicle or B- or T-labelled metaflumizone in 0.5% aqueous CMC at 30 or 1000 mg/kg bw. Each treatment group consisted of five males and five females, with the vehicle control groups being composed of one male and one female rat. Urine was collected at 0–12, 6–12 and 12–24 h post-dose and daily thereafter for 168 h. Faeces were collected at 0–12 and 12–24 h and daily thereafter for 168 h. Cage rinse was collected daily for 7 days. At termination, blood, tissues, organs and residual carcasses were collected. For both labels at the low and high dose levels, the majority of the administered radioactivity was eliminated in the faeces (89.3–111.8%), with only very small amounts eliminated in the urine (0.60–1.75%) or found in cage rinse (0.14–1.36%). In the low dose groups, most of the radioactivity was recovered within 24–48 h from the urine and faeces. In the high dose groups, excretion was slower. There was no sex difference in the excretion patterns of radioactivity. Tissues contained an average of 0.65–4.56% of the administered radioactivity. In both low and high dose groups, fat, liver, adrenals, pancreas and ovaries contained the highest concentration of radioactive residues. In most

Table 5. Recovery of radiolabel in excreta and tissues of rats at 168 h after a single oral dose of metaflumizone

Excreta/tissue	Recovery of radiolabel (% of administered dose)							
	Dose level (mg/kg bw)							
	30				1000			
	B-label		T-label		B-label		T-label	
	M	F	M	F	M	F	M	F
Urine	0.73	0.60	1.19	0.77	1.57	1.03	1.75	1.29
Cage rinse	0.14	0.14	0.29	0.31	0.63	1.24	1.36	0.80
Faeces	95.04	94.40	89.25	88.57	111.76	103.46	89.90	92.30
Tissues	2.35	2.55	1.57	2.67	0.88	4.56	0.65	2.25
Total	98.26	97.69	92.31	92.32	114.84	110.29	93.66	96.64

From Afzal & Zulalian (2002)

F, female; M, male

cases, female tissues contained higher residues than male tissues, which was more obvious in the high dose groups. The total mean recovery in urine, cage rinse, faeces and tissues from the excretion balance study ranged from 92.3% to 114.8% (Table 5).

Accumulation study: Three groups of rats were treated orally by intubation for 14 consecutive days with a 0.5% aqueous CMC dosing vehicle or B- or T-labelled metaflumizone in 0.5% aqueous CMC at 30 mg/kg bw. Each treatment group consisted of three males and three females, with the vehicle control groups being composed of one male and one female rat. The urine, faeces and cage rinses were collected from each animal at 24 h intervals after the first dose until sacrifice (Table 6). Three animals were sacrificed at each of the following time points: at 10, 48 and 168 h after the 14th dose in groups dosed with the B-label, and at 12, 168 and 288 h after the 14th dose in groups dosed with the T-label. Rats were sacrificed at or near T_{max} (the time to C_{max}), half C_{max} (the maximal concentration) and 1/8 C_{max}. At termination, blood, tissues, organs and residual carcasses were collected.

Fat, after repeated oral administration, contained the highest level of radioactive residue derived from both labels. Male rats dosed with the B-labelled metaflumizone showed highest residues of 153.0 mg/kg in fat 10 h post-dose. One hundred and sixty-eight hours post-dose, residues in fat had already decreased to a level of 69.0 mg/kg. Female rats dosed with 30 mg/kg bw B-label reached a maximum concentration of 180.6 mg/kg in fat at 48 h post-dose, decreasing to 95.18 mg/kg at 168 h post-dose. Within the T-label, male and female rats in general showed lower residues in fat, with a maximum concentration of 64.6 mg/kg in males and 79.3 mg/kg in females at 168 h post-dose. Residues decreased to 32.2 mg/kg and 39.0 mg/kg in males and females, respectively, 288 h post-dose (Table 7).

The radioactive residues in the erythrocytes and blood derived from T-label groups were noticeably higher than those observed in the B-label groups. After 14 days of daily dosing (with both B- and T-label) at 30 mg/kg bw, the metaflumizone concentration in muscle was up to 26 (muscle), 13 (liver), 13 (kidney), 43 (fat) and 26 (plasma) times higher than those found in a single oral dose group. However, metaflumizone concentrations in tissues of the 14-day dosing groups declined significantly with time.

Tissue and organ distribution study: Fourteen groups of rats were treated orally by intubation with a 0.5% aqueous CMC dosing vehicle or B- or T-labelled metaflumizone in 0.5% aqueous CMC. Each treatment group consisted of three males and three females, with the vehicle control groups being composed of one male and one female rat. Tissues were collected at three time points from

Table 6. Recovery of radiolabel in excreta and tissues of rats after 14 oral doses of metaflumizone at 30 mg/kg bw

Excreta/tissue	Recovery of B-label (% of administered dose)					
	Sacrifice (h post–14th dose)					
	10		48		168	
	M	F	M	F	M	F
Urine	1.44	2.71	2.45	2.89	2.32	2.48
Cage rinse	1.23	1.61	0.98	1.84	0.73	1.75
Faeces	71.47	76.25	82.48	80.68	88.09	81.04
Tissues	15.23	9.62	4.66	4.31	1.82	3.32
Total	89.36	90.19	90.57	89.72	92.97	88.59
	Recovery of T-label (% of administered dose)					
	Sacrifice (h post–14th dose)					
	12		168		288	
	M	F	M	F	M	F
Urine	1.85	1.30	2.29	1.28	2.29	1.05
Cage rinse	1.17	0.83	0.86	0.68	1.09	0.57
Faeces	82.06	79.02	87.74	88.44	88.09	89.92
Tissues	6.21	10.16	1.74	2.35	1.12	1.42
Total	91.30	91.32	92.64	92.75	92.59	92.96

From Afzal & Zulalian (2002)
F, female; M, male

Table 7. Recovery of radiolabel in selected tissues of rats after 14 oral doses of metaflumizone at 30 mg/kg bw

Sex	Time post-dose (h)	Recovery of radiolabel (mg/kg)					
		Muscle	Liver	Kidney	Fat	Blood	Plasma
B-label							
Males	10	5.59	20.26	11.39	153.03	1.32	1.64
	48	4.09	25.99	5.43	130.26	0.85	0.97
	168	1.34	13.88	2.52	69.00	0.42	0.21
Females	10	8.90	5.30	15.85	143.90	2.06	2.55
	48	5.91	26.93	7.58	180.59	1.00	0.99
	168	3.49	16.07	4.80	95.18	0.56	0.40
T-label							
Males	12	4.81	11.79	6.23	56.53	8.62	1.14
	168	1.50	4.72	3.04	64.59	6.42	0.44
	288	0.55	2.39	1.81	32.16	6.56	0.21
Females	12	6.48	23.35	12.53	58.88	12.6	2.80
	168	2.70	5.15	4.42	79.3	6.93	0.48
	288	1.56	2.43	1.96	38.95	4.90	0.23

From Afzal & Zulalian (2002)

each dose group. The specific time points were selected to cover a time point at the rising phase of the blood concentration curve, a time point near C_{max} and a time point at the mid-range of the declining phase. At termination, blood, tissues, organs and residual carcasses were collected.

For the B-label, total radioactivity in muscle, liver, kidney, blood and plasma reached maximal levels at or near the blood T_{max} in both male and female rats, irrespective of the dose. However, the concentration of radioactivity in the fat continued to increase until the termination time points of 48 h (B-label, low dose) and 36 h (B-label, high dose) post-dose, demonstrating the multicompartment system of the rat, with distribution to deeper compartments with increasing time periods. The T-label-derived radioactivity in the muscle, kidney, blood and, apparently, fat reached maximal levels at or near the T_{max} in both male and female rats, regardless of the dose. In contrast, the T-label-derived radioactivity in liver and plasma was highest at the earliest time point. Total radioactive residues of tissues, blood and plasma in the low and high dose groups are summarized in Table 8.

In a study conducted in order to investigate the bioavailability of metaflumizone at a lower dose, four male Wistar (Crl:WI(Han)) rats received ^{14}C-labelled metaflumizone (purity 95.8%; radiochemical purity >95%; benzonitrile ring label) by oral gavage at a single dose of 6 mg/kg bw in 0.5% aqueous CMC and 1% Cremophor and at a volume of 10 ml/kg bw. The animals were then placed in metabolism cages in order to collect urine and faeces at preset time intervals up to 168 h. After about 168 h, animals were sacrificed, and the following matrices were checked for remaining radioactivity: urine, faeces, gut contents, stomach contents, carcass and subcutaneous adipose tissue. For balance estimates, the cage wash was also checked for radioactivity.

The bioavailability (calculated as the sum of radioactivity found in urine, cage wash, subcutaneous adipose tissue and carcass) was 33.4% (Table 9). Because Cremophor is known for its properties as a surfactant/emulsifying agent, it most likely artificially enhanced the oral absorption of the very lipid-soluble molecule metaflumizone. Thus, the value of 33.4% most likely does not represent realistic absorption conditions (Fabian & Landsiedel, 2006).

In a subsequent study conducted in order to investigate the bioavailability of metaflumizone at realistic absorption conditions (i.e. without Cremophor), four male Wistar (Crl:WI(Han)) rats received ^{14}C-labelled metaflumizone (purity 97.2%; radiochemical purity >95%; benzonitrile ring label) by oral gavage at a single dose of 6 mg/kg bw in 0.5% aqueous CMC and at a volume of 10 ml/kg bw. The animals were then placed in metabolism cages in order to collect urine and faeces at preset time intervals up to 168 h. After about 168 h, animals were sacrificed, and the following matrices were checked for remaining radioactivity: urine, faeces, gut contents, stomach contents, blood cells and carcass. For balance estimates, the cage wash was also checked for radioactivity.

The bioavailability (calculated as the sum of radioactivity found in urine, cage wash, blood cells and carcass) was 16.8% (Table 9) (Fabian & Landsiedel, 2007a).

In a subsequent study, groups of five male Wistar (Crl:WI(Han)) rats received ^{14}C-labelled metaflumizone (purity 97.2%; radiochemical purity >95%; benzonitrile ring label) by oral gavage at a single dose of 0.73 mg/kg bw in 0.5% aqueous CMC and at a volume of 10 ml/kg bw or via feed at a single dose of 0.76 mg/kg bw. The animals were then placed in metabolism cages in order to collect urine and faeces at time intervals of 0–3, 3–6, 6–12 and 12–24 h and then at 24 h intervals up to 168 h. In addition, excreta were also collected during the dosing period of metaflumizone via feed (2 h). After about 168 h, animals were sacrificed, and the following matrices were checked for remaining radioactivity: urine, faeces, gut contents, stomach contents and carcass. For balance estimates, the cage wash was also checked for radioactivity.

The bioavailabilities (calculated as the sum of radioactivity found in urine, cage wash and carcass) of the test substance after dietary administration and administration by gavage were 23.0% and 10.8%, respectively (Table 9) (Fabian & Landsiedel, 2007b).

Table 8. Recovery of radiolabel in selected tissues of rats after a single oral dose of metaflumizone at 30 or 1000 mg/kg bw

Sex	Time post-dose (h)	Recovery of radiolabel (mg/kg)					
		Muscle	Liver	Kidney	Fat	Blood	Plasma
30 mg/kg bw							
B-label							
Males	2	0.247	3.660	0.950	0.633	0.103	0.148
	10	0.761	3.931	1.288	3.649	0.107	0.192
	48	0.174	2.537	0.542	6.828	0.037	0.052
	168[a]	0.129	1.587	0.308	4.987	0.025	0.024
Females	2	0.186	3.016	0.755	0.660	0.048	0.114
	10	0.573	3.044	1.111	3.894	0.087	0.130
	48	0.556	3.244	1.122	18.330	0.071	0.093
	168[a]	0.185	1.339	0.374	6.961	0.028	0.031
T-label							
Males	2	0.486	5.452	1.937	0.814	0.302	0.358
	12	0.684	2.704	1.547	4.916	0.333	0.256
	168[a]	0.087	0.311	0.225	3.506	0.350	0.029
	288	0.053	0.183	0.137	2.122	0.312	0.013
Females	1	0.128	4.078	0.924	0.213	0.233	0.255
	12	0.815	3.466	1.865	6.532	0.325	0.141
	168[a]	0.179	0.502	0.363	7.549	0.396	0.038
	288	0.129	0.279	0.239	4.230	0.298	0.017
1000 mg/kg bw							
B-label							
Males	0.5	0.484	10.800	2.164	0.798	0.730	1.084
	8	2.913	26.333	7.394	19.594	1.056	1.434
	36	1.682	18.685	3.277	34.686	0.371	0.422
	168[a]	2.290	22.830	4.474	73.271	0.554	0.730
Females	1	0.808	11.698	2.782	3.135	0.728	1.067
	4	2.063	26.872	7.801	12.291	1.115	1.639
	36	3.838	28.014	6.484	56.005	0.706	1.023
	168[a]	3.953	36.175	10.008	93.219	1.151	1.308
T-label							
Males	2	1.725	23.907	6.328	3.871	3.264	3.432
	48	3.669	14.196	7.567	85.694	6.313	1.753
	168[a]	1.445	7.322	4.212	56.144	20.733	1.692
	288	0.321	1.653	1.186	13.616	6.658	0.247
Females	1	0.785	22.099	5.682	3.249	3.750	4.678
	12	2.666	16.464	5.945	33.280	4.618	2.369
	168[a]	2.285	15.745	8.954	31.721	29.090	3.413
	288	0.868	2.293	1.595	31.596	3.968	0.209

From Afzal & Zulalian (2002)

[a] Data from separate test group (excretion balance study), $N = 5$.

Table 9. Recovery of radiolabel in excreta and tissues of male rats at 168 h after a single dose of metaflumizone (data from three studies combined)

Excreta/tissue	Recovery of radiolabel (% of administered dose)			
	Dose level (mg/kg bw), method of administration			
	6.0, gavage + Cremophor	6.0, gavage	0.73, gavage	0.76, diet
Urine	3.91	1.46	1.69	2.83
Faeces	67.94	74.79	85.55	65.77
Cage wash	0.24	0.05	0.27	0.61
Blood cells	nd	0.01	nd	nd
Stomach/stomach contents	0.04	0	0.01	0.01
Gut/gut contents	1.90	0.12	0.63	1.27
Carcass	27.29	15.29	8.79	19.57
Subcutaneous adipose tissue	1.95	nd	nd	nd
Recovery	103.25	91.71	96.95	90.06
Bioavailability	33.39	16.81	10.76	23.00

From Fabian & Landsiedel (2006, 2007a,b)
nd, not determined

In a study carried out to determine the adipose tissue concentrations after repeated exposure, 60 female Sprague-Dawley (Crl:CD) rats were administered metaflumizone (purity 96.9%) in 0.5% aqueous CMC by gavage at a dose level of 30 mg/kg bw per day for 28 days and at a volume of 10 ml/kg bw. The experiments were carried out with two different radiolabels: the ^{14}C-trifluoromethoxyphenyl-labelled (T-label; radiochemical purity 98.7%) and the ^{14}C-benzonitrile ring–labelled (B-label; radiochemical purity 98.8%) metaflumizone. Three animals each were sacrificed 2, 7, 14, 21, 28, 30, 35, 42, 49 and 63 days after first dosing. After sacrifice, remaining radioactivity was measured in adipose tissue and from day 28 also in liver, kidney, blood cells and plasma. The dose level and the selected time points for the analysis of tissue concentrations of radioactivity were based on the results obtained in a pilot study (Beimborn & Leibold, 2004a).

For the T-labelled metaflumizone (Table 10), mean concentrations of radioactivity in adipose tissue reached a maximum level of about 1022 mg/kg after 21 days, which was 1 week before the end of the dosing period. Adipose tissue concentrations of radioactivity were about 400 mg/kg and 500 mg/kg 1 and 3 days post-dosing (i.e. on study days 28 and 30), indicating that test substance uptake into adipose tissue had levelled off. During the following 33 days post-dosing (i.e. at study day 63), mean tissue concentrations of radioactivity declined in two phases to levels below 70 mg/kg, with an initial and terminal half-life of 5.2 days and 14.6 days, respectively. Radioactivity concentrations did not increase further in the fourth week of dosing, indicating that a maximum level had been reached in the adipose tissue. Concentrations of radioactivity in the additional tissues monitored during the post-dosing period were generally lower than those in adipose tissue: at least 1 order of magnitude lower in liver, kidney and blood cells and at least 2 orders of magnitude lower in plasma.

For the B-labelled metaflumizone (Table 11), the mean concentration of radioactivity in adipose tissue reached its maximum level of 918 mg/kg at the end of the dosing period. During the last application week, the radioactivity concentration in adipose tissue increased to a minor extent compared with the preceding application week, indicating that concentrations were approaching a steady state. After having reached peak levels, mean concentrations declined in two phases during the following 35 days post-dosing (i.e. at study day 63) to less than 70 mg/kg, with an initial and terminal

Table 10. Tissue concentrations of radiolabel (T-label) in female rats after repeated oral doses of metaflumizone at 30 mg/kg bw per day for 28 days

Time (study days)	Tissue concentrations of radiolabel (mg/kg)				
	Adipose tissue	Plasma	Blood cells	Liver	Kidney
2	17.93	nd	nd	nd	nd
7	126.35	nd	nd	nd	nd
14	187.07	nd	nd	nd	nd
21	1022.37	nd	nd	nd	nd
28	402.58	3.31	36.79	36.02	26.93
30	516.59	5.91	62.70	41.49	41.07
35	75.74	1.36	45.32	9.84	9.08
42	305.68	1.42	16.64	17.96	12.19
49	105.91	0.59	17.04	8.00	5.24
63	69.23	0.53	12.47	5.28	5.10
Initial half-life (days)	5.2	6.2	2.4	2.3	2.4
Terminal half-life (days)	14.6	46.3	17.9	25.6	22.3

From Beimborn & Leibold (2004b) and Fabian (2004a)
nd, not determined

Table 11. Tissue concentrations of radioactivity (B-label) in female rats after repeated oral doses of metaflumizone at 30 mg/kg bw per day for 28 days

Time (study days)	Tissue concentrations of radiolabel (mg/kg)				
	Adipose tissue	Plasma	Blood cells	Liver	Kidney
2	22.95	nd	nd	nd	nd
7	121.09	nd	nd	nd	nd
14	317.69	nd	nd	nd	nd
21	760.95	nd	nd	nd	nd
28	918.11	6.67	5.35	107.91	59.07
30	466.40	2.66	2.22	43.71	31.21
35	239.22	1.27	1.67	28.32	14.85
42	129.83	0.76	1.31	17.71	8.61
49	139.99	0.59	0.66	14.96	7.54
63	68.21	0.36	3.31	5.49	5.12
Initial half-life (days)	2.1	1.6	1.5	2.2	1.5
Terminal half-life (days)	17.0	11.4	15.9	19.7	11.6

From Beimborn & Leibold (2004b) and Fabian (2004a)
nd, not determined

half-life of 2.1 days and 17.0 days, respectively. Concentrations of radioactivity in additional tissues monitored during the post-dosing period were generally lower than those in adipose tissue—at least 1 order of magnitude lower in liver and kidney and at least 2 orders of magnitude lower in plasma and blood cells—and declined with similar half-lives as had been found in the adipose tissue (Beimborn & Leibold, 2004b; Fabian, 2004a).

Additionally, an estimation of kinetic parameters for the accumulation and depuration of metaflumizone in fat was performed using the statistical program ModelMaker. A one-compartment model was used, and a constant exposure via the gastrointestinal tract was assumed. This simplification is permissible with repeated-dose administration, as the equilibration between the central compartment and the peripheral compartment (fat) and the elimination from the peripheral compartment are much slower than the absorption from the gastrointestinal tract, thus determining the overall kinetic behaviour. Using this assumption, the half-life in fat was 8.59 days for the B-label and 9.09 days for the T-label (Kohl, 2004a,b).

1.2 Biotransformation

In the toxicokinetics study of Afzal & Zulalian (2002) described above, the radioactivity released by extraction and acid hydrolysis from various matrices of different dose groups was analysed by radio high-performance liquid chromatography (HPLC) for the unchanged parent compound and metabolites. Bile and urine were analysed from the biliary excretion study phase. Within the excretion balance phase, faeces, muscle, liver, kidney, fat and plasma were analysed. The metabolic patterns of the same matrices, except faeces, were also investigated during the accumulation and tissue and organ distribution phase.

Excretion balance study: Radio HPLC analysis of the metaflumizone-derived residues from tissues, faeces and plasma of the excretion balance study phase showed that unchanged parent was the major component of the residues. Other components of the residues were minimal and not further investigated. Highest concentrations of unchanged parent were found in fat, accounting for 96–99% of the total radioactive residue (TRR), with levels between 3.43 and 7.38 mg/kg in the low-dose treatment groups and between 30.55 and 82.0 mg/kg in the high-dose treatment groups. In kidney, the concentration of metaflumizone amounted to 0.19–0.33 mg/kg and 2.00–7.61 mg/kg for the low and high dose groups, respectively. In muscle, there was no discernible metaflumizone concentration for the low-dose treatment group, and the concentration was 0.90–3.30 mg/kg (41–84% of the TRR) for the high-dose treatment group. In plasma, the concentration of unchanged parent amounted to 0.02 mg/kg (46–90% of the TRR) for the low-dose treatment group and 0.30–1.03 mg/kg (18–78% of the TRR) for the high-dose treatment group.

Accumulation study: Radio HPLC analysis of samples from the accumulation experiments showed that unchanged metaflumizone was the major component of the TRR in tissues and plasma. The other components of the radioactive residue in the tissues and plasma were minimal, irrespective of the sex or position of the radiolabel. The highest concentrations of unchanged parent were found in fat at all sampling intervals (96.0–99.5% of the TRR), irrespective of the sex or position of the radiolabel.

Biliary excretion study: Within the biliary excretion study, bile and urine were analysed. The absorption of metaflumizone from the gastrointestinal tract was low, but the absorbed material was extensively metabolized, and the metabolites were excreted in the bile and urine. No parent was found in the urine or bile. Biliary metabolites identified included M320I04, M320I06, M320I10, M320I11, M320I12, M320I13, M320I18, M320I19, M320I20 and M320I21. The identification rate in bile samples from the B-label was 58–89% of the TRR, whereas 20–33% of the TRR from bile samples from the T-label could be identified. In urine, metabolites M320I05, M320I06, M320I10, M320I11, M320I13, M320I14, M320I15, M320I16 and M320I17 were identified. In urine, the identification rate in samples of both labels and different dose groups was between 75% and 96% of the TRR. Amounts expressed as a percentage of dose are summarized in Tables 12 and 13.

Tissue and organ distribution study: Muscle, liver, kidney, fat and plasma from the tissue and organ distribution study phase were analysed. Unchanged parent was identified as the major component of the extracted tissue and plasma residue from male and female rats treated with either a

Table 12. Quantification of metabolites in bile of rats after a single oral dose of metaflumizone at 30 or 1000 mg/kg bw

Metabolite	Amount of metabolite (% of administered dose)							
	M		F		M		F	
	Sampling interval (h)							
	0–12	12–24	0–12	12–24	0–12	12–24	0–12	12–24
	Label (group), dose							
	B-label (B-Lo-B), 30 mg/kg bw				B-label (B-Hi-B), 1000 mg/kg bw			
M320I04	0.04	0.13	0.02	0.08	0.02	0.03	0.02	0.02
M320I06	0.51	0.05	0.58	0.02	0.02	0.01	0.01	0.00
M320I10	1.35	0.21	0.89	0.04	0.62	0.06	0.29	0.02
M320I11	0.41	0.09	0.26	0.03	0.14	0.02	0.07	0.01
M320I12	0.05	0.07	0.03	0.05	0.05	0.01	0.02	0.01
M320I13	0.08	0.02	0.08	0.01	0.03	0.00	0.02	0.00
Total	2.90	0.80	2.30	0.40	1.10	0.20	0.60	0.10
Total (ppm)	*26.29*	*7.38*	*18.22*	*3.35*	*354.24*	*60.56*	*130.99*	*17.55*
	Label (group), dose							
	T-label (B-Lo-T), 30 mg/kg bw				T-label (B-Hi-T), 1000 mg/kg bw			
M320I18	0.03	0.00	0.02	0.00	0.02	0.00	0.01	0.00
M320I19 + M320I20	0.05	0.05	0.04	0.01	0.03	0.01	0.02	0.01
M320I21	0.07	0.04	0.04	0.04	0.02	0.00	0.01	0.01
Total	0.50	0.30	0.30	0.20	0.20	0.10[a]	0.10	0.10[a]
Total (ppm)	*3.75*	*1.60*	*2.04*	*2.65*	*57.23*	*28.34*	*36.32*	*13.41*

From Afzal & Zulalian (2002)
ppm, parts per million
[a] The composite bile contains <0.10% of the oral dose. For calculation purposes, 0.1% was used.

single oral low or high dose of metaflumizone. In muscle (B- and T-label), fat (B- and T-label) and liver (T-label) samples, only parent was identified. Liver samples from the B-label also showed the metabolite M320I04 at amounts of 0.01–0.06% of the administered dose. In kidney samples, unchanged parent was also the major component. Only in kidney from the T-label (high dose) could the metabolite M320I07 be identified, at up to 0.01% of the administered dose. In plasma samples, a higher number of metabolites were identified. The prominent plasma metabolite was M320I07. Minor metabolites included M320I04 and M320I21.

Metabolic pathway: Metaflumizone was metabolized via hydroxylation of the aniline or benzonitrile ring and hydrolysis of the central hydrazine carboxamide group to yield the aniline derivatives and phenacylbenzoylnitrile derivatives. The trifluoromethoxyaniline was acylated and further oxidized to form derivatives of malonic and oxalic acids. The ring hydroxylated derivatives were readily conjugated with sulfate or glucuronic acid. Glycine conjugation occurred at the carboxyl group of the cyanobenzoic acid, whereas glutathione conjugation occurred by displacement of one of the fluorine atoms of the trifluoromethyl or trifluoromethoxy group to form *S*-(*N*-(*N*-γ-glutamyl))-cysteinyl-glycyl conjugates and subsequent hydrolysis to cysteine conjugates. The proposed metabolic pathway of metaflumizone in the rat can be seen in Figure 1.

Table 13. Quantification of metabolites in urine of rats after a single oral dose of metaflumizone at 30 or 1000 mg/kg bw

Metabolite	Amount of metabolite (% of administered dose)							
	M		F		M		F	
	Sampling interval (h)							
	0–12	12–24	0–12	12–24	0–12	12–24	0–12	12–24
	Label (group), dose							
	B-label (B-Lo-B), 30 mg/kg bw				B-label (B-Hi-B), 1000 mg/kg bw			
M320I06	0.02	0.02	0.01	0.00	0.01	0.00	0.01	0.01
M320I10	0.01	0.02	0.12	0.04	0.02	0.07	0.15	0.03
M320I11	0.02	0.01	0.04	0.01	0.00	0.01	0.02	0.01
M320I13	0.14	0.03	0.18	0.03	0.07	0.01	0.08	0.03
Total	0.20	0.10[a]	0.40	0.10[a]	0.10	0.10[a]	0.30	0.10[a]
Total (ppm)	*1.85*	*0.30*	*3.13*	*0.35*	*50.27*	*12.61*	*169.31*	*10.86*
	Label (group), dose							
	T-label (B-Lo-T), 30 mg/kg bw				T-label (B-Hi-T), 1000 mg/kg bw			
M320I05	0.08	0.02	0.12	0.04	0.02	0.01	0.02	0.00
M320I14	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.00
M320I15	0.02	0.01	0.02	0.00	0.01	0.00	0.00	0.00
M320I16	0.02	0.00	0.01	0.00	0.13	0.05	0.17	0.07
M320I17	0.12	0.04	0.11	0.04	0.06	0.04	0.06	0.02
Total	0.30	0.10	0.30	0.10[a]	0.30	0.10	0.30	0.10[a]
Total (ppm)	*4.01*	*1.20*	*2.17*	*0.35*	*81.73*	*12.42*	*63.12*	*10.01*

From Afzal & Zulalian (2002)
ppm, parts per million
[a] The composite urine contains <0.10% of the oral dose. For calculation purposes, 0.1% was used.

2. Toxicological studies

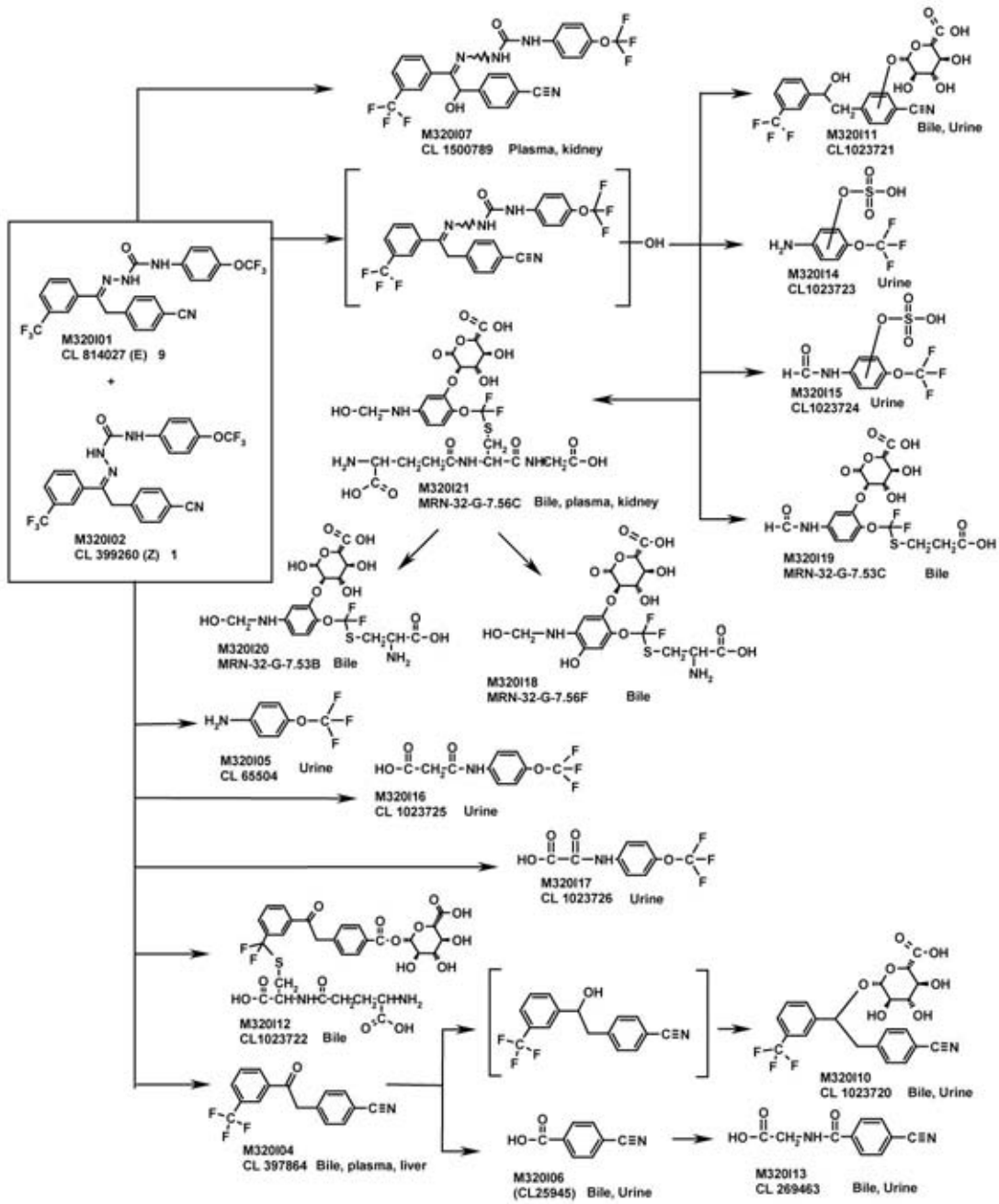
2.1 Acute toxicity studies

(a) Lethal doses

In an acute oral toxicity study, groups of five fasted CD-1 (Crl:CD-1(ICR)BR) mice of each sex were administered metaflumizone (purity 96.3%) in 0.5% aqueous CMC by gavage at a single dose of 5000 mg/kg bw and at a constant volume of 20 ml/kg bw. All animals survived, and no clinical signs were noted in the test animals throughout the 14-day study period. There were no gross pathological findings observed in animals that were sacrificed at termination. The oral median lethal dose (LD_{50}) was greater than 5000 mg/kg bw for male and female mice (Lowe, 2001b).

In an acute oral toxicity study, groups of five fasted Sprague-Dawley (Crl:CD(SD)IGS BR) rats of each sex were administered metaflumizone (purity 96.3%) in 0.5% aqueous CMC by gavage at a single dose of 5000 mg/kg bw and at a constant volume of 20 ml/kg bw. All animals survived, and no clinical signs were noted in the test animals throughout the 14-day study period. There were no gross pathological findings observed in animals that were sacrificed at termination. The oral LD_{50} was greater than 5000 mg/kg bw for male and female rats (Lowe, 2001a).

Figure 1. Proposed metabolic pathway of metaflumizone in the rat



In an acute dermal toxicity study, groups of five Sprague-Dawley (Crl:CD(SD)IGS BR) rats of each sex were administered metaflumizone (purity 96.3%) at a single dose of 5000 mg/kg bw. The test substance, a fine powder, was placed as received on a 10 cm × 10 cm, two-ply porous gauze covering and evenly distributed over a 6 cm × 6 cm area on the gauze. The gauze was immediately placed on the dorsal area of the site with tap water. Mortality was limited to one male rat that was found dead 15 min post-dosing. The death did not appear to be treatment related because there were no clinical signs of toxicity or mortality observed in the remaining animals during the 14-day study period. There were no gross pathological findings observed in animals that were sacrificed at termination and the animal that died prematurely. The dermal LD_{50} was greater than 5000 mg/kg bw for male and female rats (Lowe, 2001c).

Table 14. Summary of acute oral, dermal and inhalation toxicity studies on metaflumizone

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw) or LC_{50} (mg/l)	Reference
Mouse	Crl:CD-1(ICR)BR	M & F	Oral	96.3	>5000	Lowe (2001b)
Rat	Sprague-Dawley (Crl:CD(SD)IGS BR)	M & F	Oral	96.3	>5000	Lowe (2001a)
Rat	Sprague-Dawley (Crl:CD(SD)IGS BR)	M & F	Dermal	96.3	>5000	Lowe (2001c)
Rat	Wistar (CrlGlxBrlHan:WI)	M & F	Inhalation	96.9	>5.2	Ma & Leibold (2002)

F, female; M, male

In an acute inhalation toxicity study, groups of five Wistar (CrlGlxBrlHan:WI) rats of each sex were exposed (head–nose only) to a dust aerosol of metaflumizone (purity 96.9%) at a limit concentration of 5.2 mg/l for 4 h. The observation period lasted for 14 days. Measurements of particle size distribution showed mass median aerodynamic diameters (MMADs) of 3.1–4.1 μm. No mortality occurred at the tested concentration. Clinical signs of toxicity comprised attempts to escape during hour 0 of the exposure and visually accelerated respiration, squatting posture and smeared fur. The latter findings were observed from hour 1 of exposure until and including study day 6. The mean body weights of the male animals increased throughout the study period. The mean body weights of the female animals decreased during the first post-exposure observation week but increased during the second week. General symptoms of the gross pathology were grey-red discoloration of all lung lobes in all animals necropsied at termination of the post-exposure observation period. Under the conditions of the study, the median lethal concentration (LC_{50}) for male and female rats after dust inhalation was greater than 5.2 mg/l (Ma & Leibold, 2002).

The above acute toxicity studies are summarized in Table 14.

(b) Dermal and ocular irritation and dermal sensitization

In a study on skin irritation potential, 500 mg of pulverized metaflumizone (purity 96.3%) moistened with distilled water was applied to shorn dorsal skin of three male New Zealand White rabbits under a semioccluded dressing for 4 h. Skin reactions were scored at 1, 24, 48 and 72 h post-treatment. No signs of systemic toxicity or mortality were observed during the study period. No signs of erythema or oedema were observed during the conduct of the study. The individual mean scores at the 24, 48 and 72 h observations for erythema and oedema were 0.0 and 0.0, respectively, for each of three animals. It was concluded that metaflumizone is non-irritating to rabbit skin (Boczon, 2001a).

In a study on the eye irritation potential of metaflumizone, 100 ∝l (equivalent to 38 mg) of pulverized metaflumizone (purity 96.3%) was instilled 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 h post-instillation. Twenty-four hours after instillation, the treated eyes were rinsed with tap water. No mortality or signs of systemic toxicity were observed during the study period. No signs of corneal opacity or iris effects were observed in any of the test animals during the study. Slight conjunctival irritation was present in two test animals at the 1 h observation period. This slight conjunctival irritation resolved in one animal by the 24 h observation and in the other animal by the 48 h observation. The third animal showed slight conjunctival irritation beginning at 24 h, which resolved by 48 h. The slight conjunctival irritation in all three animals was observed as slight redness, with no chemosis or ocular discharge. The individual average scores at the 24, 48 and 72 h observations for corneal opacity and iris effects were 0.0 and 0.0, respectively, for all three test animals. The individual average scores for conjunctival redness were 0.0, 0.3 and 0.3 for all three test animals; for chemosis, the respective scores were 0.0, 0.0 and 0.0. It was concluded that metaflumizone is non-irritating to slightly irritating to rabbits' eyes (Boczon, 2001b).

Table 15. Summary of skin and eye irritation and skin sensitization studies on metaflumizone

Species	Strain	Sex	End-point	Purity (%)	Result	Reference
Rabbit	New Zealand White	M	Skin irritation	96.3	Not irritating	Boczon (2001a)
Rabbit	New Zealand White	M	Eye irritation	96.3	Non-irritating to slightly irritating	Boczon (2001b)
Guinea-pig	Dunkin Hartley (Hsd Poc:DH)	F	Skin sensitization (Magnusson and Kligman maximization test)	96.9	Not sensitizing	Gamer & Leibold (2002a)

F, female; M, male

The skin sensitization potential of metaflumizone (purity 96.9%) was investigated in female Dunkin Hartley (Hsd Poc:DH) guinea-pigs using a Magnusson and Kligman maximization test. The test substance concentrations for the main test were selected based on the results of the pretest in three females for epicutaneous induction with test substance preparations in 25% and 50% aqueous CMC and in two females for intradermal induction with substance preparations in 5% aqueous CMC. The intradermal induction was performed with a 5% test substance preparation in 1% CMC solution in doubly distilled water or 5% test substance preparation in Freund's adjuvant/0.9% aqueous sodium chloride solution (1:1) and the epicutaneous induction with a 50% test substance preparation in 1% CMC solution in doubly distilled water. For the challenge, a 25% test substance preparation in 1% CMC solution in doubly distilled water was chosen. The study was initiated with two control groups (10 female animals each) and one test group (20 female animals). The intradermal induction was performed on day 0, and the epicutaneous induction on day 7. A challenge was carried out 14 days after the epicutaneous induction. The intradermal induction caused moderate and confluent to intense erythema and swelling at the injection sites of the test substance preparations in all test group animals. After the epicutaneous induction, incrustation, partially open (caused by the intradermal induction), could be observed, in addition to intense erythema and swelling in all test group animals. The challenge with a 25% test substance preparation did not cause any skin findings either in control group animals or in test group animals 24 and 48 h after removal of the patches. As no borderline results were observed, a second challenge was not performed. It was concluded that metaflumizone does not have a sensitizing effect on the skin of the guinea-pig in the maximization test under the test conditions chosen (Gamer & Leibold, 2002a).

The above dermal and ocular irritation and dermal sensitization studies are summarized in Table 15.

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a non-GLP study, groups of five male and five female CD-1 mice were given diets containing metaflumizone (purity not reported) at concentrations of 0, 50, 200 and 800 parts per million (ppm), equal to 0, 10, 42 and 101 mg/kg bw per day, for 28 days. Food consumption and body weight data were recorded, as well as organ weights and pathology of selected organs.

At 800 ppm, five out of five males and four out of five females died prior to the end of week 2. Correlating body weight losses were observed for animals at 800 ppm, some of which also demonstrated clinical signs of toxicity (ataxia and convulsions) prior to death. Following 1 week of treatment at 800 ppm, mean food consumption was markedly decreased by 55% for males and by 37% for females, compared with controls. Similarly, following 1 week of treatment at 200 ppm, mean

food consumption was markedly decreased by 32% for males, compared with controls. Slight reductions in mean food consumption were noted for males at 200 ppm for the remainder of the study as well as for females at 200 ppm during the entire study. Correlating body weight losses occurred for males and females at 200 ppm during week 1, which contributed to the overall statistically decreased body weight gains for males and females at 200 ppm. Histopathological changes were noted in the spleen (atrophy) and ovaries (delay in sexual maturation) at the dietary concentrations of 200 and 800 ppm; however, these changes were considered secondary to nutritional deficiencies, as evidenced by a substantial decrease in body weight gain and decreased food consumption at those dietary concentrations.

The no-observed-adverse-effect level (NOAEL) was 50 ppm, equal to 10 mg/kg bw per day, based on reductions in body weight, body weight gain and food consumption at 200 ppm, the next highest concentration tested, which may be related to the poor palatability observed with the diet admixture containing metaflumizone (Hess, 2007e).

In a non-GLP study, groups of five male and five female CD-1 mice were given diets containing metaflumizone (purity not reported) at concentrations of 0, 10, 20 and 40 ppm, equal to 0, 2.0, 4.3 and 8.2 mg/kg bw per day, for 28 days. An additional five animals in the control and high dose groups were used for investigation following a 28-day recovery period. Food consumption and body weight data were recorded, organ weights were measured and pathology of selected organs was performed. Only slight, not statistically significant decreases in body weight, body weight gain and food consumption were observed at 40 ppm. These slight changes were not considered to be adverse. No organ weight changes and no treatment-related histopathological changes were observed up to 40 ppm. No treatment-related mortalities occurred and no clinical signs of toxicity were noted at 40 ppm. For the recovery animals, no differences were observed for body weight, body weight gain, food consumption or organ weights, compared with controls.

The NOAEL was 40 ppm, equal to 8.2 mg/kg bw per day, the highest dose tested (Hess, 2007f).

Rats

Groups of five male and five female Sprague-Dawley CD rats were given diets containing metaflumizone (purity 94.9%) at concentrations of 0, 250, 1000, 5000 or 10 000 ppm, equal to 0, 27.2, 86.4, 417.5 and 798.1 mg/kg bw per day in males and 0, 23.8, 83.2, 360.6 and 783.0 mg/kg bw per day in females, for 4 weeks. Food consumption, body weight, body weight gain, clinical signs, clinical chemistry, haematology and full necropsy were performed in this investigation.

No animals died. At 250 ppm, dorsal hair loss was seen in one female. At 1000 ppm and above, thin build and hair loss on the head and dorsal body in both sexes were recorded. In addition, females receiving 10 000 ppm exhibited pallor, hunched posture and transient brown staining of the forelimbs and nose. Body weight gain and food consumption were markedly reduced at 1000 ppm and higher, and there was also an increase in the amount of food actively discarded by these animals. At 250 ppm, there was lower weight gain and food consumption during the early part of the study only.

Treatment-related haematological findings in week 4 included low packed cell volumes and haemoglobin concentrations in animals receiving 5000 or 10 000 ppm; low mean cell haemoglobin and mean cell volumes in males receiving 1000, 5000 or 10 000 ppm; low mean cell haemoglobin and mean cell haemoglobin concentrations (MCHC) in females receiving 10 000 ppm; low neutrophil and lymphocyte counts in females receiving 1000, 5000 or 10 000 ppm; and slightly prolonged prothrombin times at all concentrations in females.

Biochemical changes in the plasma in week 4 comprised high total bilirubin concentrations in animals receiving 5000 or 10 000 ppm; high total cholesterol concentrations at 250 ppm and above in females and at 1000 ppm and above in males; high glucose and urea concentrations at 1000 ppm

and higher in females; and high albumin concentrations in males at 10 000 ppm and low globulin concentrations in females at 1000 ppm and above, resulting in high albumin to globulin ratios in females at 5000 ppm and in males and females receiving 10 000 ppm. At 1000 ppm and higher, females had low calcium concentrations.

After 4 weeks of treatment, relative adrenal weights were increased in males at 1000 ppm and above and in females at 10 000 ppm. Ovary and uterus weights were decreased at 250 ppm and higher. Macroscopic examination revealed thin build in animals given 1000 ppm and above. Histopathological changes after 4 weeks of treatment included atrophy of the splenic white pulp for males and females at 5000 ppm and higher, reduced leukocytes in the splenic white pulp in females given 5000 ppm and in males and females given 10 000 ppm, and atrophic/undeveloped uterus and reduced corpora lutea in the ovaries of females at 1000 ppm and above.

The NOAEL was less than 250 ppm, equal to less than 23.8 mg/kg bw per day, based on lower weight gain and food consumption during the early part of the study (Bolton, 2000).

In a non-GLP study, groups of 15 male and 15 female Sprague-Dawley rats were given diets containing metaflumizone (purity not reported) at concentrations of 0, 50, 100, 200 or 400 ppm, equal to 0, 3.8, 7.2, 13.7 and 26.3 mg/kg bw per day, for up to 90 days. Following 28 days of treatment, 5 rats of each sex per group were sacrificed, whereas the remaining 10 rats of each sex per group were sacrificed after 90 days of treatment. Daily clinical observations were performed, and body weight and food consumption were determined weekly. Haematological evaluations were conducted after 1, 2 and 3 months. Clinical chemistry was conducted at 1 month and at 3 months. Organ weights were determined, and histopathological evaluations were conducted on all animals.

No mortality or clinical signs of toxicity were noted during the study. Weekly mean food consumption, mean body weights and mean body weight gains were statistically significantly decreased at all dietary concentrations. During the first week, mean food consumption was reduced in males by 34%, 48% and 58% at 100, 200 and 400 ppm, respectively. In females during the first week, the mean food consumption was decreased by 50%, 59% and 61% at 100, 200 and 400 ppm, respectively. By the end of the 13-week treatment period, total mean food consumption was reduced in males by 20%, 30% and 45% at doses of 100, 200 and 400 ppm. In females, the total reduction at week 13 was 28%, 36% and 45% of control at doses of 100, 200 and 400 ppm, respectively.

Subsequent marked decreases in mean body weight gain were observed at all dietary concentrations. Specifically, during the first week, mean body weight gain was decreased by 53% and 83% for males at 100 and 200 ppm. Moreover, an actual mean body weight loss (0.2 g) occurred at 400 ppm. In females during the first week, actual mean body weight losses of 0.5, 12.3 and 17.4 g were observed at 100, 200 and 400 ppm, respectively. By week 13, cumulative mean body weight gain was reduced in males by 27%, 35% and 57% at 100, 200 and 400 ppm, respectively. In females, the total reduction at week 13 was 32%, 36% and 56% at the same respective dietary concentrations (Table 16).

Haematological evaluations indicated slight decreases in total white blood cells and lymphocytes at 400 ppm, compared with controls. No other significant haematological changes were recorded. Absolute ovary and uterus weight changes were noted in females of the 400 ppm dose group at the 28-day and 90-day sacrifice. Histopathologically, changes in the ovaries (visibly small, with reduced number of corpora lutea), uterus (visibly small and hypoplastic, with no evidence of cyclic activity) and liver (decreased hepatocyte lipid vacuolation) were noted in female rats at 200 and 400 ppm that are consistent with delayed sexual maturation and with nutritional deficiency, as evidenced by a substantial decrease in body weight gain and decreased food consumption at those dietary concentrations.

Table 16. Summary of selected findings in a 28-day/90-day dietary toxicity study in rats

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	50	100	200	400	0	50	100	200	400
Feed consumption (g)										
- Week 1	157	138**	104**	82**	67**	124	101**	61**	50**	48**
- Week 4	204	193	172**	150**	113**	142	131	109**	96**	77**
- Week 13	200	186	167**	156**	126**	132	126	104**	97**	84**
Body weight (g)										
- Week 1	201	188**	166**	148**	136**	158	147*	121**	109**	106**
- Week 4	362	341**	308**	282**	234**	229	216	194**	181**	158**
- Week 13	566	522*	447**	411**	324**	303	276*	243**	235**	203**
Body weight gain (g)										
- Week 1	66	51**	30**	11**	−0.2**	35	23**	−0.5**	−12**	−17**
- Total gain	429	383**	312**	275**	185**	178	151*	199**	114**	80**

From Hess & Hastings (2007)
* $P < 0.05$; ** $P < 0.01$

The NOAEL for this 28-day/90-day dietary toxicity study was less than 50 ppm, equal to less than 3.8 mg/kg bw per day, based on decreases in body weight gain and food consumption in both sexes at 50 ppm, the lowest dose tested (Hess & Hastings, 2007).

Because a NOAEL was not determined in the previous 28-day/90-day dietary toxicity study, an additional (non-GLP) study was conducted. Groups of five male and five female Sprague-Dawley rats were given diets containing metaflumizone (purity not reported) at concentrations of 0, 10, 20 and 40 ppm, equal to 0, 1.1, 2.2 and 4.3 mg/kg bw per day, for 28 days. Food consumption and body weight data were recorded, as well as clinical chemistry, organ weights and pathology of selected organs.

Reductions in mean food consumption, mean body weights and mean body weight gains were noted for male and female rats at 40 ppm. Specifically, decreased mean food consumption was observed in males and females during weeks 1 and 2 of the study. These reductions were statistically significant in females, resulting in decreases of 16% and 13% at weeks 1 and 2, respectively. A lesser, but non-statistically significant, decrease of 7% occurred for 40 ppm males at weeks 1 and 2. In the 40 ppm group, body weight gains during the first 2 weeks of treatment were reduced by approximately 12% and 16% for males and females, respectively, and overall body weight gains were reduced by approximately 4% and 13% for males and females, respectively. These slight reductions in mean food consumption and subsequent mean body weight gains may be partially caused by poor palatability observed with the diet admixture containing metaflumizone. No organ weight changes and no treatment-related histopathological changes were observed. No mortalities occurred, no clinical signs of toxicity were noted and no changes in the clinical chemistry data were seen at 40 ppm.

The NOAEL was 20 ppm, equal to 2.2 mg/kg bw per day, based on reductions in body weights, body weight gains and food consumption at 40 ppm, the highest concentration tested (Hess, 2007d).

In order to investigate the effect of metaflumizone on dietary palatability, two male Sprague-Dawley rats were fed either control feed or a diet containing 400 ppm of metaflumizone (purity not reported) on alternating days for 4 days in a non-GLP study. Body weight and food consumption were measured daily. When control feed was provided on day 1 and day 3, both animals consumed

Table 17. Body weight, body weight change and food consumption in a palatability study in rats

Study day	Body weight (g)	Body weight change (g)	Food consumption (g), control feed	Food consumption (g), 400 ppm metaflumizone
0	352	—	—	—
1	360	+8.0	27.5	—
2	344	−16.0	—	16.5
3	354	+10.0	28.0	—
4	345	−9.0	—	13.0
Overall mean			55.5	29.5

From Hess (2007a)

Table 18. Mean daily food consumption in a "free choice" palatability study in rats

Study day	Mean daily food consumption (g)					
	Test group			Control group		
	Jar A (control)	Jar B (400 ppm)	Total consumption	Jar A (control)	Jar B (control)	Total consumption
1	20.0	2.6	22.6	15.0	8.4	23.4
2	24.4	0.8	25.2	12.8	13.8	26.6
3	25.8	2.4	28.2	12.4	12.8	25.2
4	23.4	1.6	25.0	11.4	15.2	26.6
5	24.8	0.4	25.2	8.2	18.8	27.0
Days 1–5	—	—	126.2	—	—	128.8

From Hess (2007b)

25–30 g of food per day. In contrast, when treated feed was provided on day 2 and day 4, both animals consumed only 9–13 g of food per day. The overall mean food consumption for both animals when provided with treated feed was approximately 47% less than the overall mean food consumption for both animals when provided with control feed (Table 17). In addition, both male rats demonstrated mean body weight gains of 8–10 g, respectively, when fed control diets on days 1 and 3. In contrast, when fed 400 ppm metaflumizone-treated diet on days 2 and 4, both animals demonstrated actual body weight losses of 16.0 and 9.0 g, respectively (Hess, 2007a).

In order to investigate the effect of metaflumizone on dietary palatability, a "free choice" non-GLP study was conducted in which a test group of five male Sprague-Dawley rats was offered one jar (A) of control feed and one jar (B) of a diet containing 400 ppm of metaflumizone (purity not reported) each for 5 days. Further, a control group of five male rats was offered two jars of control feed for 5 days. The positions of the respective two jars were changed daily in each individual cage. Body weight and food consumption were measured daily. The "free choice" animals that were offered control and treated feed containing 400 ppm metaflumizone exhibited a strong preference for the control feed by avoiding the treated feed. The mean daily food consumption was between 20 and 25.8 g of control feed and between 0.4 and 2.6 g of treated diet (Table 18). The control rats demonstrated highly consistent daily mean food consumption between 23.4 and 27.0 g without a preference for either jar A or jar B (Hess, 2007b).

In a further non-GLP study conducted to investigate the effect of metaflumizone on dietary palatability and food consumption, groups of five male Sprague-Dawley rats received metaflumizone

(purity not reported) by oral gavage at dose levels of 0, 6, 16 and 28 mg/kg bw per day for 7 days. These oral gavage doses are equivalent to compound intakes during week 1 of the 28-day/90-day feeding study in rats at 0, 50, 200 and 400 ppm dietary concentrations (Hess & Hastings, 2007). Only body weight gain and food consumption were recorded during this preinvestigation.

Following 1 week of oral gavage treatment, comparable overall mean food consumption (144.8, 137.2, 146.8 and 145.6 g) was calculated for male rats at respective dose levels of 0, 6, 16 and 28 mg/kg bw per day of metaflumizone. Similarly, following 1 week of treatment, mean body weight gains were comparable between control and treatment animals. Specifically, overall weight gains were 52.6, 49.6, 54.0 and 55.4 g for the rats at 0, 6, 16 and 28 mg/kg bw per day of metaflumizone. These results support the hypothesis that reductions in food consumption and subsequent decreased body weight gains observed in the feeding studies are caused by the poor palatability of the diet admixture containing metaflumizone (Hess, 2007c).

Groups of five male and five female Sprague-Dawley (Crl:CD(SD)BR) rats received metaflumizone (purity 95.1%) by oral gavage at dose levels of 0, 100, 500 and 1000 mg/kg bw per day for 28 days, whereas an additional five rats of each sex were dosed with the test substance at either 0 or 1000 mg/kg bw per day for 13 weeks. Owing to a marked decrease in body weight during the first week of the study, the high dose was reduced to 100 mg/kg bw per day for the 13-week animals and continued at this dose level for the remainder of the study period. The test substance was administered as 0.5% aqueous CMC solution at a constant volume of 10 ml/kg bw. The rats were observed daily for signs of overt toxicity, morbidity and mortality. Individual body weights and food consumption data were recorded weekly during the study period. Samples for haematological and clinical chemistry examinations were collected from all surviving rats at 28 days (interim sacrifice) and at termination (13 weeks). At the interim and terminal sacrifices, all surviving animals were subjected to gross necropsy, and selected organs were weighed. Samples of selected tissues were processed for histopathological evaluation from all surviving test animals.

Survival was unaffected by administration of the test substance. In the 28-day phase, food consumption was statistically significantly decreased in both sexes at 1000 and 500 mg/kg bw per day and slightly (statistically non-significantly) decreased in both sexes at 100 mg/kg bw per day (Table 19). In the 13-week phase, food consumption for females at 1000/100 mg/kg bw per day was slightly (and frequently statistically significantly) reduced, with corresponding males showing no treatment-related changes after the dose was reduced to 100 mg/kg bw per day. At 28 days, body weights and body weight gains were statistically significantly decreased at 1000 mg/kg bw per day (−43% for males and −51% for females) and at 500 mg/kg bw per day (−36% for males and −46% for females). Slight (statistically non-significant) decreases in body weight gain (−12% for males and −23% for females) were observed at 100 mg/kg bw per day. In the 13-week phase, body weights were lower, and body weight gains for females at the 1000/100 mg/kg bw per day dose were slightly reduced (−13%), with corresponding males showing no treatment-related changes.

At 28 days, slight (statistically non-significant) decreases in total erythrocytes, haemoglobin and haematocrit were observed for females at 1000 and 500 mg/kg bw per day. These decreases correlated with the microscopic finding of extramedullary haematopoiesis of the spleen observed at these doses. No treatment-related haematological changes were noted in either sex at 100 mg/kg bw per day. At 13 weeks, no treatment-related haematological changes were noted in either sex in the 1000/100 mg/kg bw per day dose group at study termination.

At 28 days, serum aspartate aminotransferase (ASAT) activity was statistically significantly increased in females at 1000 mg/kg bw per day and slightly increased (not statistically significantly) in 500 mg/kg bw per day females. Male rats in the 1000 and 500 mg/kg bw per day groups showed slight, statistically non-significant increases in ASAT activity. Although cholesterol concentration was significantly increased in males at 1000 mg/kg bw per day and in females in all metaflumizone-

Table 19. Summary of selected findings in a 28-day toxicity study in rats

Parameter	Males				Females			
	Dose level (mg/kg bw per day)							
	0	100	500	1000	0	100	500	1000
Food consumption (g)								
- Days 0–7	159.5	141.4	111.8*	102.8*	128.3	109.0*	70.4*	75.4*
- Days 7–14	178.2	163.6	115.4*	125.0*	138.1	122.2	89.6*	86.8*
- Days 14–21	195.8	190.8	118.4*	153.6*	156.4	124.6	88.2*	89.0*
- Days 21–28	203.6	189.2	159.0*	141.4*	156.2	122.4	118.0*	95.0*
Body weight (g)								
- Day 0	153.2	155.8	154.8	153.0	120.1	121.8	121.2	120.8
- Day 7	205.5	194.2	174.0*	164.5*	151.6	141.2	119.6*	121.0*
- Day 14	258.7	246.0	206.4*	195.0*	176.8	164.8	142.2*	137.0*
- Day 21	305.8	292.4	229.8*	220.4*	198.6	179.8	150.2*	151.8*
- Day 28	345.1	324.8	276.8*	249.6*	217.0	196.0	173.6*	167.8*
Body weight gain (g)								
- Days 0–7	52.3	38.4	19.2*	11.5*	31.5	19.4	−1.6*	0.2*
- Days 7–14	53.2	51.8	32.4*	36.2*	25.2	23.6	22.6	21.8
- Days 14–21	47.2	46.4	23.4*	25.4*	21.8	15.0	8.0	14.8
- Days 21–28	39.2	32.4	47.0	29.2*	18.4	16.2	8.0	14.8
- Days 0–28	191.9	169.0	122.0*	94.0*	96.9	74.2	52.4*	55.4*
(% change from control)	(—)	(−11.9)	(−36.4)	(−51.0)	(—)	(−23.4)	(−45.9)	(−42.8)
Absolute liver weight (g)	11.72	11.30	10.09	9.41	7.96	7.29	6.26*	5.97*
Relative liver weight (%)	3.64	3.88	4.19*	4.32*	4.10	4.18	4.31	4.14
Absolute testes/ovaries weight (g)	3.49	2.91*	3.04	2.75*	0.163	0.129	0.099*	0.086*
Relative testes/ovaries weight (%)	1.08	1.01	1.26	1.29	0.084	0.074	0.068	0.059*
Hepatocellullar hypertrophy	0/5	0/5	2/5	4/5	0/5	0/5	2/5	4/5
Spleen, increased extramedullary haematopoiesis	0/5	0/5	2/5	3/5	0/5	0/5	3/5	5/5

From Fischer (2002)
* $P < 0.05$

treated groups, no dose–response relationship was evident. At 13 weeks, no treatment-related clinical chemistry changes were noted in either sex in the 1000/100 mg/kg bw per day dose group at study termination.

At 28 days, relative liver weights were statistically significantly increased in male rats in the 1000 and 500 mg/kg bw per day dose groups, with corresponding females showing a slight, statistically non-significant increase. The increases in relative liver weight correlated with the microscopic finding of hepatocellular hypertrophy in these same treatment groups. At 13 weeks, no treatment-related organ weight changes were noted in either sex in the 1000/100 mg/kg bw per day dose group at study termination. No treatment-related macroscopic changes were noted in either sex in the 1000, 500, 100 or 1000/100 mg/kg bw per day dose groups at either the 28-day or 13-week time points of the study.

At 28 days, extramedullary haematopoiesis was observed in the spleen in the 500 and 1000 mg/kg bw per day treatment groups for both males and females. Additionally, hepatocellular

hypertrophy was observed in both sexes in the 1000 and 500 mg/kg bw per day treatment groups. No treatment-related microscopic pathological changes were observed in either sex in the 100 mg/kg bw per day dose group at study termination. At 13 weeks, a minimal increase in haemosiderin pigment was observed in the spleen of two out of five females at 1000/100 mg/kg bw per day. This finding may have been related to splenic extramedullary haematopoiesis that was observed in the 1000 mg/kg bw per day group at 28 days and could have been a result of receiving 1000 mg/kg bw per day during the first week of the study.

The NOAEL was less than 100 mg/kg bw per day, based on decreased body weight gain observed over the 28-day treatment period at this dose level and the slight decrease in food consumption and body weight gain observed in females at 1000/100 mg/kg bw per day over the 13-week period (Fischer, 2002).

Groups of 10 male and 10 female Sprague-Dawley CD (Crl:CD (SD) IGS BR) rats received metaflumizone (purity 96.3%) by oral gavage at dose levels of 0, 30, 60 and 300 mg/kg bw per day for a period of 90 days. Control animals received the vehicle, 0.5% CMC in distilled water, at the same dose volume (10 ml/kg bw) as administered to the treated animals. Beginning the first day of week 3, the dose level of the high dose group females was lowered to 200 mg/kg bw per day because of an adverse effect on body weight. The study was conducted as part of a chronic toxicity and carcinogenicity study (see section 2.3).

Study animals were checked twice daily for mortality and clinical signs. Clinical examinations were performed pretest and once weekly. Body weight and food consumption measurements were obtained pretest and weekly during the treatment period. Ophthalmoscopic examinations were conducted on all animals pretest and for selected animals prior to the 90-day sacrifices. Blood samples were collected for haematology, coagulation, clinical chemistry and urinalysis at termination (10 of each sex per group). Neurobehavioural evaluations, motor activity and functional observational battery (FOB) were conducted on selected animals (10 of each sex per group) pretest and at week 12. After at least 90 days of treatment, animals were sacrificed, selected organs were weighed and organ to body weight and organ to brain weight ratios were calculated. Complete macroscopic examinations and histopathological evaluation of tissues were conducted on all animals. Analyses conducted during the treatment period confirmed that dosing suspensions of the appropriate concentration were administered.

No test substance–related mortality occurred during the 3 months of the study. Mean body weights and body weight gains as well as food consumption of females administered 300 mg/kg bw per day were significantly decreased compared with control values during the first 2 weeks of the study (Table 20). The difference in mean body weight from control at week 2 was −16%, and the difference in mean body weight gain from control was −71%. Therefore, the dose level was lowered from 300 to 200 mg/kg bw per day beginning week 3. At the end of 3 months, differences from control in mean body weight and body weight gain were −12% and −27%, respectively. The only test substance–related microscopic finding was an increased incidence of central lobular hepatocellular hypertrophy in the liver of 9 out of 10 males (minimal to slight), which correlated with slightly but non-statistically increased absolute and relative liver weights. All other parameters evaluated, including clinical findings, ophthalmoscopic examinations, neurobehavioural studies, haematology, coagulation, clinical chemistry and urinalysis data as well as macroscopic examinations, did not reveal any adverse test substance–related effects.

The NOAEL was 60 mg/kg bw per day, based on reduced food consumption and decreased body weights and body weight gains in females at 300/200 mg/kg bw per day (Kelly, 2003b).

Dogs

Groups of five male and five female purebred Beagle dogs were administered metaflumizone (purity 96.9%) via gelatine capsules at dose levels of 0, 6, 12, 30 and 60/40/30 mg/kg bw per day for 12 months. Owing to pronounced clinical findings in the high dose group, the initial dose level (60 mg/

Table 20. Summary of selected findings in a 90-day toxicity study in rats

Parameter	Males				Females			
	Dose level (mg/kg bw per day)							
	0	30	60	300	0	30	60	300/200[a]
Body weight (g)								
- Week 0	207	206	206	206	163	163	163	164
- Week 2	306	311	306	292**	212	208	203**	177**
- Week 6	428	438	428	411	264	257	254**	229**
- Week 12	518	534	531	498	296	288	285*	262**
Absolute liver weight (g)	13.97	14.88	15.71	15.09	8.24	8.23	7.95	7.60
Relative liver weight (%)	2.87	2.97	2.95	3.04	2.99	3.12	3.17	3.29*
Hepatocellular hypertrophy[b]								
- Minimal	0	0	2	4	0	0	0	0
- Slight	0	0	0	5	0	0	0	0

From Kelly (2003b)
* $P < 0.05$; ** $P < 0.01$
[a] Dose levels were lowered beginning week 3.
[b] Incidence (number of animals with hepatocellular hypertrophy out of 10 animals).

kg bw per day) was lowered to 40 mg/kg bw per day on study day 49 and to 30 mg/kg bw per day on study day 245. Additional clinical, clinicochemical and haematological examinations were performed after 3 months of study duration. As no satellite group was used, no pathological examinations were performed after 3 months. Food consumption of the animals was determined each working day, and their body weights were determined once a week. The animals were examined at least once each working day for any signs of toxicity, and a check for any moribund or dead animals was made either twice a day (Mondays to Fridays) or once a day (Saturdays, Sundays and on public holidays). Moreover, detailed clinical examinations, including open field observations, were conducted prior to the start of the administration period and weekly thereafter. Clinical chemistry and haematological examinations as well as urinalyses were carried out once before the beginning of test substance administration, after about 7 weeks and after approximately 3, 6, 9 and 12 months of test substance administration. Ophthalmological examinations were carried out 20 days before the beginning of the administration period, after about 3 months (day 85) and towards the end of the administration period (study day 363). All animals were subjected to gross pathological assessment, followed by histopathological examinations.

In the 60/40/30 mg/kg bw per day group, one male and two females were euthanized on day 57, and one male and one female were euthanized on days 250 and 226, respectively. In the 30 mg/kg bw per day group, two females were euthanized on study days 215 and 237. These dogs were prematurely sacrificed for humane reasons due to clinical findings (poor general state of health, ataxia, lateral position, vomitus) indicative of severely impaired state of health. The animals showed pronounced impairment of food consumption—even cessation of food consumption in some cases—and consequently decreased body weights/body weight gains (including body weight loss) prior to premature sacrifice. In the 12 mg/kg bw per day group, a male dog was found dead on study day 317. This death was considered to be unrelated to the test substance, as pathology revealed a rupture/perforation of the jejunum as the direct cause of death.

Retarded body weight gain or body weight loss was observed in two males and four females in the 60/40/30 mg/kg bw per day group, after impairment of food consumption and food conversion efficiency in these animals. In general, females were affected with higher incidence and to a higher extent than male animals. In the 30 mg/kg bw per day group, retarded body weight gain (not leading

to premature sacrifice) was seen in two males; one did not gain weight during the entire study period, and the other demonstrated slight retarded body weight gain. In females, four animals demonstrated retarded body weight gain or body weight loss, which led to premature sacrifice in two animals, as described above.

The mean red blood cell count, haemoglobin levels and haematocrit for both sexes in the 30 and 60/40/30 mg/kg bw per day groups were lower than the values for the controls over the course of the study. These lower values were reflected in a lower calculated MCHC for both sexes in these groups. Although the MCHC values for both sexes in the 6 and 12 mg/kg bw per day group were statistically different from those of the control at various times during the study, a dose–response relationship was not readily evident. In the clinical chemistry examination, the serum total bilirubin levels for both sexes in the 30 and 60/40/30 mg/kg bw per day groups were elevated at various times during the treatment period (Table 21).

Table 21. Summary of selected findings in a 3-month/12-month toxicity study in dogs

Parameter	Males					Females				
	Dose level (mg/kg bw per day)									
	0	6	12	30	60/40/30	0	6	12	30	60/40/30
No. sacrificed	0	0	0	0	2	0	0	0	2	3
No. found dead	0	0	1	0	0	0	0	0	0	0
Body weight (kg), day −1	11.1	11.5	11.2	11.3	10.9	10.2	10.1	10.1	10.3	10.3
Body weight gain (kg)										
- Day 56	2.1	2.4	2.6	2.1	1.6	2.4	1.9	1.9	1.8	0.1**
- Day 203	3.3	3.9	3.9	2.0	3.7	4.1	3.4	2.7	0.7**	1.2*
- Day 364	4.0	5.0	5.3	3.0	5.4	4.8	4.4	3.2	3.8	2.7
Haemoglobin (mmol/l)										
- Day 173	10.2	9.4	9.4	8.7*	8.9*	10.5	10.2	9.4	9.7	9.5
- Day 271	10.3	9.8	9.7	9.1	9.0	9.8	10.1	9.5	9.0	9.3
- Day 362	10.3	10.2	10.1	9.3	9.2	10.8	9.9	9.5*	9.2*	9.3
MCHC (mmol/l)										
- Day 173	21.6	21.0**	21.0*	20.3**	20.4*	21.2	20.5**	20.6**	20.3**	19.9*
- Day 271	21.9	21.1	21.0*	20.6**	21.0*	21.6	20.8**	21.0**	20.6*	20.7
- Day 362	20.8	20.6	20.6	20.1	20.3	21.8	20.8**	20.8**	20.3*	20.5
Hypochromasia[a]										
- Day 173	0	0	0	3/5	1/4	0	0	0	0	3/3
- Day 271	0	0	0	3/5	0	0	0	0	0	0
- Day 362	0	0	0	3/5	2/3	0	0	0	1/3	0
Total bilirubin (µmol/l)										
- Day 173	3.11	3.97	3.97	5.38**	4.43**	3.56	4.58	4.16	5.55**	6.08*
- Day 271	3.31	4.64	4.10	5.70	4.07	4.01	4.03	4.71	5.36	5.97
- Day 362	3.70	4.21	4.56	6.82**	4.82	3.65	4.93	4.69	5.31*	6.03
Body weight (kg), terminal	15.18	16.54	16.03	14.38	16.73	15.12	14.66	13.42	14.67	13.40
Absolute liver weight (g)	387	469**	423	422	467	400	385	357	408	422
Relative liver weight (%)	2.56	2.84*	2.64	2.94*	2.80	2.66	2.63	2.65	2.81	3.19
Absolute spleen weight (g); mean	33.6	39.0	43.2	50.4	46.9	36.4	38.6	40.0	43.6	51.1

Table 21 (contd)

Parameter	Males					Females				
	Dose level (mg/kg bw per day)									
	0	6	12	30	60/40/30	0	6	12	30	60/40/30
Absolute spleen weight (g); individual values	33.07	26.93	41.05	34.01	43.20	36.63	40.11	42.58	37.48	58.80
	38.94	43.52	46.85	44.70	46.54	33.31	38.66	35.99	42.68	43.37
	30.03	41.45	38.43	31.67	50.91	36.32	34.01	42.78	50.73	
	38.95	40.90	46.65	56.93		35.83	40.83	41.98		
	26.90	42.32		84.65		39.83	39.63	36.70		
Relative spleen weight (%); mean	0.221	0.236	0.270	0.345	0.280	0.243	0.265	0.301	0.298	0.381
Relative spleen weight (%); individual values	0.209	0.164	0.263	0.254	0.279	0.213	0.279	0.266	0.268	0.384
	0.235	0.256	0.2	0.306	0.269	0.214	0.254	0.313	0.328	0.377
	0.198	0.245	97	0.229	0.293	0.286	0.281	0.354	0.298	
	0.251	0.245	0.251	0.385		0.233	0.254	0.278		
	0.210	0.270	0.268	0.553		0.271	0.256	0.296		
Liver; haemosiderosis[b]	2	3	3	5	4	2	3	5	5	4
- Grade 1: minimal	2	2	2	0	2	1	3	3	2	2
- Grade 2: slight	0	1	1	4	2	1	0	2	3	2
- Grade 3: moderate	0	0	0	1	0	0	0	0	0	0
Kidney; pigment storage[b]	5	5	5	5	4	3	4	4	4	3
- Grade 1: minimal	4	1	0	0	0	3	2	2	3	2
- Grade 2: slight	1	1	3	1	1	0	2	2	0	0
- Grade 3: moderate	0	2	1	1	0	0	0	0	1	1
- Grade 4: marked, severe	0	1	1	3	3	0	0	0	0	0

From Kaspers et al. (2004a)
* $P < 0.05$; ** $P < 0.01$
[a] Incidence (number observed/number examined).
[b] Number observed out of 5 examined.

In the necropsy examination, the mean absolute and relative spleen weights were greater for both sexes in the 12, 30 and 60/40/30 mg/kg bw per day groups. However, a dose–response relationship was not always apparent. Evaluation of individual relative spleen weights indicated that in males at 12 mg/kg bw per day, the mean of the study (0.270%) is essentially in the range of the upper limit for relative spleen weights in historical control animals (0.268%, based on five studies), with three of four dogs being within this upper range and one dog (0.297%) slightly exceeding the upper range. For female dogs at 12 mg/kg bw per day, the mean of the study (0.301%) is at the upper limit for relative spleen weights in historical control animals (0.303%, based on five studies), with three of five dogs being below this upper range. The relative spleen weight (0.313%) for another female dog is essentially on the point of the upper range, whereas the remaining female dog had a relative spleen weight of 0.354%, which clearly exceeds the upper range. Owing to the lack of any microscopic correlation in splenic histopathology with the slightly increased relative spleen weight of one male and one female dog at 12 mg/kg bw per day, this finding was not considered to be of potential adverse toxicological or biological significance.

Although the mean absolute and relative liver weights were greater for males in all of the treatment groups, no dose–response effect was evident. In the histopathological examination, atrophy of the testes, prostate gland and thymus was noted for the animals in the 30 and 60/40/30

mg/kg bw per day groups that were euthanized during the study. In the kidneys, a greater degree of pigment storage in the tubular epithelium (probably lipofuscin) was reported for the males in all of the treatment groups with evidence of a dose-related effect. For the females, this effect was less evident. In the liver, microscopic examination revealed a tendency towards increased incidences of minimal to slight haemosiderosis in Kupffer cells. No histopathological effects were noted in the spleen.

The NOAEL for male and female Beagle dogs in this combined 3-month/12-month toxicity study was 12 mg/kg bw per day, based on clinical signs of poor general state of health and premature sacrifice, decreased food consumption, body weights and body weight gains, decreased MCHC and increased total bilirubin in both sexes and increased hypochromasia in males at 30 mg/kg bw per day and above (Kaspers et al., 2004a).

(b) Dermal application

Rats

Groups of 10 male and 10 female Wistar rats were administered metaflumizone (purity 96.9%) dermally for 3 months (6 h/day, 5 days/week, semiocclusive dressing) at dose levels of 0, 100, 300 and 1000 mg/kg bw per day. The test substance was administered as 0.5% aqueous CMC solution at a volume of 4 ml/kg bw. Food consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Additionally, clinical examinations were carried out daily. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. Ophthalmological examinations were performed before and towards the end of the application period. Clinicochemical and haematological examinations were performed after 3 months, whereas urinalyses were performed after 4 weeks and after 3 months. All animals were assessed by gross pathology, followed by histopathological examinations.

No deaths resulted from the treatment. Both sexes in the 300 and 1000 mg/kg bw per day groups demonstrated reduced food consumption (−2.8% and −9.0% in males, −13.0% and −29.8% in females, respectively, compared with controls) and lower mean body weight gain (−13.3% and −15.8% in males, −32.1% and −53.4% in females, respectively, compared with controls) over the course of the study, with females being affected to a greater extent.

No treatment-related effects were noted in the ophthalmology, haematology or urinalysis examinations. The calcium, sodium and inorganic phosphate levels were elevated for the 1000 mg/kg bw per day females ($P < 0.05$ or 0.01). The mean serum cholesterol levels were increased for the 300 and 1000 mg/kg bw per day females ($P < 0.05$ or 0.01). In the necropsy examination, the mean relative liver weight of the 1000 mg/kg bw per day females was increased over that of the controls ($P < 0.01$). In the histopathology examination, an increased incidence and severity of lymphocytic necrosis were noted in the spleen of the 1000 mg/kg bw per day females (0: 3/10 versus 1000: 6/10). Reduction of the periartiolar lymphoid sheath was noted in the spleen of three of the females in the 1000 mg/kg bw per day group. In the thymus, an increased incidence and severity of starry sky cells in females of the 300 and 1000 mg/kg bw per day groups were observed (0: 5/10 versus 300: 8/10, 1000: 9/10). Lymphocytic necrosis was noted in the mesenteric lymph nodes of the 300 and 1000 mg/kg bw per day females (0: 0/10 versus 300: 3/10, 1000: 4/10). Atrophy of the mandibular lymph nodes was noted for the 300 and 1000 mg/kg bw per day females (0: 0/10 versus 300: 2/10, 1000: 2/10). Vacuolation of the zona fasciculata in the adrenal cortex was noted for the 1000 mg/kg bw per day females (0: 0/10 versus 1000: 6/10).

The NOAEL for general systemic toxicity after dermal application in rats was 100 mg/kg bw per day, based on decreased food consumption, body weight/body weight change and lymphocytic necrosis at 300 mg/kg bw per day and above. There were no substance-related signs of local irritation at dose levels up to 1000 mg/kg bw per day (Kaspers, 2004; Kaspers et al., 2004b).

(c) Inhalation

Rats

Groups of five male and five female Wistar rats were nose-only exposed to dust aerosol atmospheres containing metaflumizone (purity 96.9%) for 6 h per working day for 28 days (20 exposures) at target concentrations of 30, 100 and 700 mg/m^3 and MMADs of 1.7, 2.1 and 3.3 µm, respectively. A control group (five rats of each sex) was exposed to clean air using the same technical procedures as for test substance exposure. The general state of health was assessed twice daily on workdays and once per day on weekends or holidays. On exposure days, clinical observation was performed before, during and after exposure. During the acclimation period and on post-exposure days, clinical findings were recorded once each working day. Moreover, body weight development, food consumption and food conversion efficiency were monitored during the whole study period. Haematological and clinicochemical examination and urinalysis were performed towards the end of the study. A complete necropsy, including weighing of selected organs and gross pathological evaluation, was performed. Histopathology was performed on selected organs and tissues and on organs showing abnormal weight.

Four weeks of nose-only inhalation exposure to a dust aerosol of metaflumizone led to pronounced reduced food consumption and a corresponding marked reduction in body weight in the high (700 mg/m^3) and middle concentration (100 mg/m^3) groups. The poor general state of health of the animals led to treatment-related reduced survival in the highest concentration group. Additionally, treatment-related local effects indicating some irritation from the presence of dust particles were observed in the nasal cavity and lungs at these concentrations. The maximum tolerated dose was clearly exceeded in males and females of the high concentration group and in females of the middle concentration group owing to increased mortality and/or severe body weight loss.

In addition to inanition associated with exposure to the test substance, the restraint stress associated with nose-only exposure is regarded as the triggering stress factor for the animals. The combination of restraint stress and inanition resulting in severe body weight loss caused a number of indirect (secondary) effects. These changes, which occurred in various organs (e.g. thymus, spleen, mediastinal and mesenteric lymph nodes, adrenal glands, glandular stomach, ovaries, uterus, bone marrow, prostate and seminal vesicles), are considered to be related to the poor physical state of the animals and stress, because they were accompanied by marked body weight loss in the middle and high concentration groups. There were no treatment-related effects observed at the low concentration (30 mg/m^3).

The no-observed-adverse-effect concentration (NOAEC) was 30 mg/m^3, based on significant reduced food consumption and corresponding significant reduced body weights and local irritation in the nasal cavity and lungs at the next higher concentration of 100 mg/m^3 and above (Ma et al., 2004b).

Ten male and 10 female Wistar rats per test group were whole-body exposed to dust aerosol atmospheres containing metaflumizone (purity 95.8%) for 6 h per working day for about 28 days (20 exposures) at a target atmosphere concentration of 30 mg/m^3 and an MMAD of 1.8 µm. A concurrent control group (10 rats of each sex) was exposed to clean air using the same technical procedures as for test substance exposure. A second control group (10 rats of each sex) that was maintained in the animal room, exposed to clean air and not handled was used in order to enable a differentiation between potential restraint stress–induced effects and potential substance-related effects observed in the previous study using a nose-only procedure (Ma et al., 2004b). The general state of health was assessed twice daily on workdays and once per day on weekends or holidays. On exposure days, clinical observation was performed before, during and after exposure. During the preflow period and on post-exposure days, clinical findings were recorded once each working day. Moreover, body weight gain, food consumption and food conversion efficiency were monitored

Table 22. Summary of selected findings in a 28-day inhalation toxicity study in rats[a]

Parameter	Males			Females		
	Concentration (mg/m^3)					
	00	0	30	00	0	30
Body weight, day 28 (g)	293.5	291.9	267.5** (2)	184.0	176.8	160.1** (2)
Body weight change, days 0–28 (g)	+47.6	+48.2	+20.1** (2)	+25.0	+18.2** (1)	−2.2** (2)
Food consumption, day 28 (g)	nd	22.8	22.0	nd	17.1	13.1** (2)
Cholesterol (mmol/l)	1.88	1.99	2.43** (2)	1.81	1.57	3.20** (2)
Chloride (mmol/l)	112.8	113.6	112.9	110.1	111.8	108.4** (2)
Urea (mmol/l)	8.07	8.40	7.98	7.57	7.80	6.86** (2)
Creatinine (µmol/l)	70.0	70.7	70.0	68.5	72.6	64.3** (2)
Albumin (g/l)	42.21	41.57	43.45* (2)	42.83	42.71	40.02* (2)
Inorganic phosphate (mmol/l)	2.63	2.81	2.54	2.44	2.23* (1)	2.95** (2)
Absolute thymus weight (mg)	303	319	318	385	312* (1)	269
Relative thymus weight (%)	0.106	0.111	0.121	0.215	0.180	0.173
Absolute adrenal weight (mg)	78	79	78	74	69	75
Relative adrenal weight (%)	0.027	0.028	0.030	0.041	0.040	0.048* (2)
Adrenal cortical vacuolation (*N*)	2	2	2	2	0	9

From Ma et al. (2004a)

nd, not determined; * $P < 0.05$; ** $P < 0.01$

[a] Statistical analysis was done comparing group 0 (control/inhalation chamber) with group 00 (control/animal room), which is referred to as (1) in the table, and comparing the 30 mg/m^3 group with group 0, which is referred to as (2) in the table.

during the whole study period. Haematological and clinicochemical examinations of numerous parameters were performed at the end of the study. A complete necropsy, including weighing of selected organs and gross pathological evaluation, was performed. Histopathology was performed on lymph nodes, thymus, spleen and adrenal gland.

Four weeks of handling and chamber housing led to markedly reduced body weight gain, slightly reduced food consumption and decreased absolute and relative thymus weights in females (Table 22).

Four weeks of inhalation of a dust aerosol at a target atmosphere concentration of 30 mg/m^3 of metaflumizone led to moderately decreased food consumption and a corresponding marked reduction in body weight gain in male and female animals that were whole-body exposed to the test article. However, there was a lack of systemic toxicity noted except for several indirect effects involving slight clinicochemical changes. The increase in relative adrenal weights in treated females corresponded to a slight increase in incidence of adrenal cortical vacuolation (Table 22). This is also considered to be a secondary effect due to the stress caused by body weight loss.

Importantly, the actual exposure of the animals was higher than the target atmosphere concentration of 30 mg/m^3, due to additional dermal and oral exposure that occurs by whole-body exposure (Ma et al., 2004a).

As a part of the 28-day inhalation toxicity study described above (Ma et al., 2004a), the residue levels of metaflumizone in serum of rats were measured at the end of the study. The analysis of the individual isomers showed that little conversion of the *E* isomer to the *Z* isomer occurred in the animals. The *Z* isomer was found only in small amounts compared with the *E* isomer. Traces of the *E* isomer of metaflumizone (0.005–0.007 mg/kg) were detected in several samples of untreated

Table 23. Summary of selected findings in an 18-month carcinogenicity study in mice

Parameter	Males				Females			
	Dose level (mg/kg bw per day)							
	0	100	250	1000	0	100	250	1000
Body weight, week 76 (g)	37.6	38.1	38.1	35.6*	32.2	32.0	31.3	31.8
Body weight change, weeks 0–76 (g)	12.5	12.4	13.0	10.4**	11.9	12.0	10.8	11.7
Spleen, number examined	65	65	65	65	65	65	65	65
Spleen, brown pigment[a]								
- Slight	0	3	2	13	3	6	2	23
- Moderate	0	0	0	6	0	1	2	4
- Total	0	3	2	19*	3	7	4	27*
Lungs, number examined	65	64	65	65	65	65	65	65
Visceral pleura: inflammation[a]	5	7	1	15*	7	3	3	9
Pleural thickening/adhesion[a]	5	5	0	17*	3	1	1	5
Mediastinal tissue, number examined	3	3	3	15	3	4	2	6
Mediastinal tissue: granulomatous inflammation[a]	0	2	0	15*	1	0	0	4

From Kelly (2003a)
* $P < 0.05$; ** $P < 0.01$
[a] Number of mice (out of number examined) in which finding observed.

rats. These findings were most likely caused by carryover or contamination of solvents or laboratory equipment. Residues in the treated group were more than 100-fold higher, so this contamination had no influence on the results from the treated rats. In the treated group, total residues of metaflumizone in serum were in the range from 0.90 to 1.69 mg/kg in male rats and from 2.33 to 4.52 mg/kg in female rats. Residues were significantly higher in female rats than in male rats. The average results were 1.17 mg/kg for males and 3.65 mg/kg for females. These results correspond to the small but slightly more pronounced clinical effects found in the females (Tilting, 2004).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 65 male and 65 female CD-1 mice (Crl:CD-1(ICR)BR) received metaflumizone (purity 96.3%) by oral gavage at dose levels of 0, 100, 250 and 1000 mg/kg bw per day for a period of 18 months. Control animals received the vehicle, 0.5% CMC in distilled water, at the same dose volume (10 ml/kg bw) as administered to the treated animals. Observations for mortality and clinical signs were conducted twice daily. Clinical examinations, body weight and food consumption measurements were performed on all animals pretest and at selected intervals during the treatment period. Haematological parameters were evaluated on 10 animals of each sex per group at 12 and 18 months (termination). After 18 months of treatment, all survivors were sacrificed, and selected organs were weighed. Complete macroscopic examinations and histopathological evaluation of selected tissues for all animals were conducted. Analyses conducted prior to and during the treatment period confirmed that the test substance preparations were stable and homogeneous and that the appropriate concentrations were administered.

There was no treatment-related effect upon the survivability of the treated animals. The mean body weight and mean body weight gain of the 1000 mg/kg bw per day males were lower than those

of the controls over the course of the study (Table 23). There was no apparent treatment-related effect on the mean food consumption or on the haematological parameters of the treated animals. In the histopathological examination, a higher proportion of animals with increased amounts of brown pigment in the spleen was noted in both sexes in the 1000 mg/kg bw per day group. Although there was an increased incidence of inflammation of the visceral pleural/capsule and pleural thickening/adhesions and the presence of granulomatous inflammation in the mediastinal tissue for the males in the 1000 mg/kg bw per day group ($P < 0.05$), these effects were attributed to injuries inflicted as a consequence of the gavage dosing regimen.

The NOAEL for oncogenicity in the mouse was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for non-neoplastic changes was 250 mg/kg bw per day, based on decreased body weight and body weight gain in males and increased incidences of brown pigment in the spleen of both sexes at 1000 mg/kg bw per day (Kelly, 2003a).

Rats

Groups of 80 male and 80 female Sprague-Dawley CD (Crl:CD (SD) IGS BR) rats received metaflumizone (purity 96.3%) by oral gavage at dose levels of 0, 30, 60 and 300 mg/kg bw per day for a period of up to 24 months. Control animals received the vehicle, 0.5% CMC in distilled water, at the same dose volume (10 ml/kg bw) as administered to the treated animals. For interim assessment, designated animals (10 of each sex per group) were sacrificed after 90 days or 12 months of treatment. Beginning the first day of week 3, the dose level of the high dose group females was lowered to 200 mg/kg bw per day because of an adverse effect on body weight. Study animals were checked twice daily for mortality and clinical signs. Clinical examinations were performed pretest and once weekly. Body weight and food consumption measurements were obtained pretest and weekly for the first 16 weeks and monthly thereafter during the treatment period. Ophthalmoscopic examinations were conducted on all animals pretest, for selected animals prior to the 90-day and 12-month sacrifices and on all survivors at study termination. Blood samples were collected for haematology, coagulation, clinical chemistry and urinalysis at months 3, 6, 9 (haematology only), 12, 18 and 24 on selected animals (10 of each sex per group). Neurobehavioural evaluations, motor activity and FOB were conducted on selected animals (10 of each sex per group) pretest and at week 12. After 90 days or 12 months of treatment, designated animals (10 of each sex per group) and all survivors at study termination were sacrificed, selected organs were weighed, and organ to body weight and organ to brain weight ratios were calculated. Complete macroscopic examinations and histopathological evaluation of tissues were conducted on all designated animals.

No test substance–related mortality occurred during the study. Survival rates were lower than 50% for all treated and control groups of males and females; however, the results of the study are nevertheless considered to be valid, as percentage survival rates for all treated groups were only slightly below 50%. Although all surviving male rats were sacrificed 4 weeks early owing to the mortality in the control group (22% survival rate), it is unlikely that any additional treatment-related tumours would develop that were not detected at the 23-month time point. Furthermore, the survival rate of the male rats was well within the historical control data for this strain in this laboratory (15%, 18%, 22% and 28% for the four doses, respectively).

The mean body weights of the males in the 300 mg/kg bw per day group and the females in the 60 and 300/200 mg/kg bw per day groups were lower than those of the control animals at various time points during the first 3 months of the treatment. The lower mean body weight persisted throughout the remainder of the study for the females at 300/200 mg/kg bw per day. Food consumption was lower for the females in the 300/200 mg/kg bw per day group during the first weeks of the study. In the haematological evaluation after 3 and 6 months of treatment, the haemoglobin concentration and red blood cell count were reduced for the males in the 300 mg/kg bw per day group, whereas the reticulocyte count was elevated after 3 months in this group. The haematology values demonstrated no consistent treatment-related effects throughout the remainder of the study. The FOB and motor activity assessments after 12 weeks of treatment did not reveal any treatment-related effects. No

Table 24. Summary of selected findings in a 24-month toxicity and carcinogenicity study in rats

Parameter	Males				Females			
	Dose level (mg/kg bw per day)							
	0	30	60	300	0	30	60	300/200[a]
% survival (no. of total survivors/ no. of potential survivors)	22 (12/55)	46** (27/59)	40* (23/57)	48* (28/58)	35 (20/57)	45 (25/55)	46 (26/57)	46 27/59)
Body weight (g)								
- Week 12	518	534	531	498	296	288	285*	262**
- Week 52	738	772	762	741	410	396	396	360**
- Week 96/104[b]	766	796	810	748	452	475	455	418
Haemoglobin (g/l), 3 months	16.3	16.2	15.8	15.5*	15.7	15.5	15.4	15.5
RBC (10^{12} cells/l), 3 months	9.23	9.38	9.10	8.77**	8.46	8.35	8.43	8.20
Haemoglobin (g/l), 6 months	16.5	15.7*	15.4**	15.3**	15.1	15.0	14.7	14.4
RBC (10^{12} cells/l), 6 months	9.53	9.13*	9.02*	8.89*	8.04	8.10	7.90	7.83
Hepatocytes; central lobular hypertrophy[c]								
- Interim sacrifice, 3 months	0/10	0/10	2/10	9/10	0/10	0/10	0/10	0/10
- Interim sacrifice, 12 months	0/10	0/10	4/10	8/10	0/10	0/10	2/10	0/10
- Unscheduled deaths	0/48	0/33	11/37	10/32	0/40	0/35	0/34	2/33
- Terminal sacrifice	0/12	0/27	3/23	22*/28	0/20	0/25	0/26	11*/27
- All animals	0/80	0/80	20*/80	49*/80	0/80	0/80	2/80	13*/80
Hepatocytes; basophilic alteration[c]								
- Interim sacrifice, 3 months	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
- Interim sacrifice, 12 months	2/10	0/10	0/10	4/10	0/10	1/10	2/10	0/10
- Terminal sacrifice	3/12	7/27	12/23	12/28	8/20	9/25	10/26	11/27
- All animals	9/80	9/80	15/80	20*/80	19/80	21/80	16/80	18/80

From Kelly (2003b)
RBC, red blood cells; * $P < 0.05$; ** $P < 0.01$
[a] Dose levels were lowered beginning week 3.
[b] Body weight, for males at week 96 and for females at week 104.
[c] Incidence (number of animals with finding/number of animals examined).

treatment-related effect was noted in the ophthalmology, clinical chemistry or urinalysis. No treatment-related effects on organ weights were evident in the necropsy examination. In the histopathological examination, centrilobular hepatocellular hypertrophy was evident for the males in the 60 and 300 mg/kg bw per day groups and in females in the 300/200 mg/kg bw per day group. Also in males, increased incidences of hepatocellular basophilic alteration were noted in the 60 and 300 mg/kg bw per day groups at the termination of the study (Table 24).

The NOAEL was 30 mg/kg bw per day, based upon increased incidences of centrilobular hepatocellular hypertrophy and hepatocellular basophilic alteration in males at 60 mg/kg bw per day. The NOAEL for oncogenicity was 300 mg/kg bw per day for males and 200 mg/kg bw per day for females, the highest doses tested (Kelly, 2003b).

2.4 Genotoxicity

In a reverse gene mutation assay in bacteria, *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2 *uvr*A) were exposed to metaflumizone (purity 96.3%) using dimethyl sulfoxide (DMSO) solvent in the presence and absence of S9 activation in two

independent sets of experiments. Triplicate plates were used per dose and per condition. Vehicle and positive controls were included in each experiment. Metaflumizone was tested up to the limit concentration of 5000 µg/plate. No cytotoxicity was observed in a preliminary cytotoxicity assay with and without metabolic activation using all tester strains. Hence, concentrations of 15, 50, 150, 500, 1500 and 5000 µg/plate were tested using the plate incorporation procedure. No cytotoxicity was observed at any dose. Precipitation of the test substance was noted at concentrations of 500 µg/plate and higher or 150 µg/plate and higher in the first and second experiments, respectively. The positive controls induced the appropriate responses in the corresponding strains. There was no elevation in the number of revertant colonies per plate in any of the tests. It was concluded that metaflumizone was not mutagenic in the bacterial strains tested, either in the presence or in the absence of metabolic activation (Wagner & Klug, 2001).

Metaflumizone (purity 96.9%) was tested in vitro for the ability to induce forward mutations in mammalian cells by assessing the mutation of the *HPRT* locus in Chinese hamster V79 cells. Two independent sets of experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay, concentrations of 156.3–5000 µg/ml were used in the original experiment both with and without metabolic activation. The confirmatory experimental concentrations of 25–800 µg/ml and 50–1200 µg/ml were used without and with metabolic activation, respectively. Ethylmethanesulfonate (EMS) and methylcholanthrene (MCA) served as positive controls in the experiments without and with metabolic activation. The cells were treated for 4 h in the experiments both without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated for about 1 week for expression of mutant cells. This was followed by a 1-week incubation of cells in selection medium containing 6-thioguanine. No increase in the mutant frequency was observed in either the original or the confirmatory studies. In contrast to this, the positive control substances EMS and MCA resulted in a marked increase in mutant frequency. Based on the results of the study, it is concluded that under the conditions of the test, metaflumizone does not induce forward mutations in mammalian cells in vitro (Engelhardt & Leibold, 2002d; Engelhardt, 2004c).

Metaflumizone (purity 96.9%) was tested in vitro for the ability to induce chromosomal and numerical aberrations in Chinese hamster V79 cells in two independent experiments in the presence and absence of metabolic activation. Based on the results of preliminary cytotoxicity assays in which substantial precipitation of the test substance was noted at concentrations of 500 µg/ml and above, concentrations of 12.5–100 µg/ml were tested for clastogenic effects with and without metabolic activation in an experiment with 4 h of treatment followed by 14 h of recovery. Vehicle (DMSO) and positive controls (cyclophosphamide [CPP] and EMS for the experiments with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Prior to cell harvest, addition of colcemid arrested cells in the metaphase. After slide preparation and staining of the cells, 200 well-spread metaphases per dose and treatment condition were analysed for chromosomal aberrations. In the experiment without metabolic activation, a statistically significant increase in the number of cells with aberrations was noted at 12.5 and 50 µg/ml. No increase was observed at 25 µg/ml, and the concentration of 100 µg/ml revealed an insufficient number of well-spread metaphases. In contrast, no effect was observed in the experiment with metabolic activation. In a second experiment without metabolic activation employing five concentrations ranging from 3.125 to 50 µg/ml, again, an increase in the incidence of aberrant cells was observed at 12.5 µg/ml and above, this time including 25 µg/ml. Based on the results of this study, metaflumizone is considered to have a clastogenic potential in vitro in Chinese hamster V79 cells in the absence of metabolic activation (Engelhardt & Leibold, 2002b; Engelhardt, 2004d).

In a micronucleus test, groups of five male Crl:NMRI mice received two oral gavage doses (24 h apart) of metaflumizone (purity 96.9%) at 0 (0.5% aqueous CMC), 500, 1000 and 2000 mg/kg bw in a volume of 10 ml/kg bw. The vehicle served as the negative control, and CPP (20 mg/kg bw)

Table 25. Summary of genotoxicity studies with metaflumizone

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* (TA98, TA100, TA1535, TA1537)	±S9 mix: 0, 15, 50, 150, 500, 1500, 5000 µg/plate	96.3	Negative	Wagner & Klug (2001)
	E. coli (WP2 *uvr*A)				
Gene mutation, *HPRT* locus	Chinese hamster V79 cells	±S9 mix: 0, 25, 50, 100, 156.3, 200, 312.5, 400, 625, 800, 1200, 1250, 2500, 5000 µg/ml	96.9	Negative	Engelhardt & Leibold (2002d)
Chromosomal aberration	Chinese hamster V79 cells	–S9 mix: 0, 3.125, 6.25, 12.5, 25, 50 µg/ml +S9 mix: 0, 25, 50, 100 µg/ml	96.9	Positive Negative	Engelhardt & Leibold (2002b)
In vivo					
Micronucleus induction	Male Crl:NMRI mice – bone marrow erythroblasts	0, 500, 1000, 2000 mg/kg bw; twice (24 h apart); intraperitoneal administration	96.9	Negative	Engelhardt & Leibold (2002c)
UDS	Primary rat hepatocytes	0, 1000, 2000 mg/kg bw; oral gavage	96.9	Negative	Engelhardt & Leibold (2003a)

S9, 9000 × *g* rat liver supernatant

and vincristine sulfate (0.15 mg/kg bw) served as positive controls. The animals were sacrificed 24 h after the last administration, the bone marrow of the two femora was prepared and 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The number of normochromatic erythrocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes was also recorded. A dose-related increase of clinical signs was observed in metaflumizone-treated mice. At 500 and 1000 mg/kg bw, squatting posture was observed up to 4 h after administration. In addition to this, piloerection and a poor general state were observed at the dose level of 2000 mg/kg bw. No signs of systemic toxicity were observed in any of the animals treated with the positive control substances or the vehicle. Two intraperitoneal administrations of metaflumizone did not lead to any increase in the number of polychromatic erythrocytes containing either small or large micronuclei. A slight inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, indicated that the test substance actually reached the bone marrow. Both of the positive control chemicals led to the expected increase in the rate of polychromatic erythrocytes containing small (CPP) or small and large (vincristine sulfate) micronuclei, thus demonstrating the sensitivity of the test system (Engelhardt & Leibold, 2002c; Engelhardt, 2004e).

In an in vivo unscheduled deoxyribonucleic acid (DNA) synthesis (UDS) test, groups of three male Wistar (CrlGlxBrlHan:WI) rats received a single oral gavage administration of metaflumizone (purity 96.9%) at 0 (0.5% aqueous CMC), 1000 and 2000 mg/kg bw in a volume of 10 ml/kg bw. As positive control, 2-acetylaminofluorene (2-AAF) was administered at a dose of 50 mg/kg bw. Hepatocytes were harvested 3 and 14 h after administration. No signs of systemic toxicity were observed in any of the animals treated with the test or control substances. No increase in the number of net nuclear grain counts or in the percentage of cells in repair was noted at any dose in hepatocytes harvested 3 h or 14 h after administration of metaflumizone. In contrast, 2-AAF treatment led to a marked increase in the number of net nuclear grains as well as the percentage of cells in repair, thus demonstrating the sensitivity of the test system. Thus, under the experimental conditions of this assay, metaflumizone is considered to be negative in the in vivo UDS assay using rat hepatocytes (Engelhardt & Leibold, 2003a; Engelhardt, 2004b).

The above genotoxicity studies with metaflumizone are summarized in Table 25.

2.5 Reproductive toxicity

(a) Multigeneration studies

In a two-generation reproductive toxicity study, groups of 25 male and 25 female Wistar (CrlGlxBrlHan:WI) rats (F_0 parental generation) received metaflumizone (purity 96.9%) as an aqueous suspension by oral gavage at doses of 0, 12, 30 and 75 mg/kg bw per day. Control animals received the vehicle, 0.5% CMC in distilled water, at the same dose volume (10 ml/kg bw) as administered to the treated animals. At least 75 days after the beginning of treatment, F_0 animals were mated to produce a first litter (F_{1A} generation pups). Mating pairs were from the same dose group. Because of excessive maternal toxicity at the 75 mg/kg bw per day dose level, together with clinical signs of poor general state of health, decreased food consumption, body weight and body weight gain, and also high pup mortality, a meaningful assessment of the reproductive toxicity potential of the test compound was impossible at this dose. Therefore, all surviving F_{1A} pups were killed on day 21 post-partum (p.p.) and examined macroscopically at necropsy (including weight determinations of brain, spleen and thymus in one pup of each sex per litter) without selecting any F_{1A} pups as the basis for the next (i.e. second) parental generation as originally scheduled. As a consequence, the dose levels were lowered, and animals were dosed at the reduced levels for a new premating period of 10 weeks and subsequently for two generations. Thus, from study day 126, the F_0 generation parental animals received the test substance at partly reduced doses (0, 12, 20 and 50 mg/kg bw per day) orally by gavage. The F_0 parental rats were treated with the test substance during a 10-week period (premating) and were then mated again with the same partner as for their first mating to produce a second litter (F_{1B} generation pups). The F_0 females were allowed to litter and rear the F_{1B} generation pups until day 4 (standardization) or day 21 after parturition.

Groups of 25 males and 25 females that were selected from the F_{1B} generation pups as F_1 parental generation were treated with the test substance at dosages of 0, 12, 20 and 50 mg/kg bw per day post-weaning, and the breeding programme was repeated to produce the F_2 generation pups (i.e. premating period followed by gestation and lactation phases for the F_2 litter). The study was terminated with the terminal sacrifice of the F_2 weanlings and F_1 adult animals.

There were no substance-related mortalities in any of the parental animals in any of the groups.

F_{1A} litter production: At the initial treatment levels of 0, 12, 30 and 75 mg/kg bw per day for the F_0 animals, poor general state of health was observed in 1 mid-dose and 11 high-dose females during premating, in 1 mid-dose and 6 high-dose females during gestation and in 5 high-dose females during lactation (Table 26). Significant reductions in female body weight and food consumption during premating, gestation and lactation were noted. High-dose maternal body weight gains were also significantly reduced during premating, and body weight gains for mid-dose females were significantly below control values during gestation days 0–7. One (4%), 0, 4 (16%) and 7 males (28%) at 0, 12, 30 and 75 mg/kg bw per day, respectively, failed to produce litters. This represented a significant reduction in the male fertility index at the high dose level (72%) compared with concurrent controls (96%) and was outside of the historical control range (84–100%). The female fertility index was also significantly reduced at the high dose level (75%) compared with concurrent controls (96%) and fell outside of the historical control range (84–100%). One (4%), 0, 4 (16%) and 6 (25%) sperm-positive females at 0, 12, 30 and 75 mg/kg bw per day, respectively, did not deliver. The mean estrous cycle length at the high dose level (5.4 days) was significantly increased compared with controls (4.2 days), mainly due to two females with cycle lengths of 14 or 16 days. The estrous cycle of one rat was unaffected when this animal received a lower dose (i.e. 50 mg/kg bw per day), and the other animal died at a later stage due to severe chronic progressive glomerulonephropathy. Thus, increased estrous cycle length is not considered to be treatment related. Sperm parameters were not evaluated.

The numbers of F_{1A} pups that died and were cannibalized during the lactation period were significantly increased at 75 mg/kg bw per day (43 died, 7 cannibalized) compared with controls (1 and

Table 26. Summary of selected findings for F_0 and F_1 parents in a two-generation reproductive toxicity study in rats

Parameter	Males				Females			
	Dose level (mg/kg bw per day)							
	0	12	30/20[a]	75/50[b]	0	12	30/20[a]	75/50[b]
F_0, mating A								
Poor general state, weeks 0–17 (*N*)	1	0	0	0	0	0	1	11
Food consumption (g/day), weeks 0–10	21.1	21.1	21.5	20.7	15.3	15.5	15.8	13.3
Body weight (g), week 10	336.9	335.3	348.5	338.2	204.8	205.3	200.6	176.1**
Body weight gain (g), weeks 0–10	222.5	221.1	234.4	224.6	105.9	105.4	101.3	76.9**
Successfully mated (*N*)	24	25	25	24	25	25	25	24
Mating index (%)	100	100	100	96	100	100	100	96
Males without progeny (*N*)	1	0	4	7	NA	NA	NA	NA
Pregnant females (*N*)	NA	NA	NA	NA	24	25	21	18*
Fertility index (%)	96	100	84	72*	96	100	84	75*
Estrous cycle length (days)	NA	NA	NA	NA	4.2	4.1	4.2	5.4*
F_0, mating B								
Poor general state, weeks 0–17 (*N*)	0	0	0	0	0	0	1	7
Food consumption (g/day), weeks 0–10	20.2	20.6	20.5	19.8	15.0	15.7	16.1	15.1
Body weight (g), week 10	413.2	412.4	429.3	423.5	239.2	241.5	237.0	221.9**
Body weight gain (g), weeks 0–10	31.5	31.3	33.1	32.9	10.3	13.4	10.5	9.9
Successfully mated (*N*)	24	25	24	24	25	25	24	24
Mating index (%)	100	100	100	100	100	100	100	100
Males without progeny (*N*)	0	0	3[c]	3[c]	NA	NA	NA	NA
Pregnant females (*N*)	NA	NA	NA	NA	25	25	21	21
Fertility index (%)	100	100	88	88	100	100	88	88
Estrous cycle length (days)	NA	NA	NA	NA	4.1	4.7	4.1	4.4
F_1, mating A								
Poor general state, weeks 0–17 (*N*)	1	1	0	0	0	0	0	0
Food consumption (g/day), weeks 0–10	20.9	20.5	22.1	21.5	16.6	16.5	16.8	16.6
Body weight (g), week 10	334.0	333.6	360.6*	339.5	216.7	214.1	220.7	218.3
Body weight gain (g), weeks 0–10	264.9	268.1	290.0	271.4	151.3	150.8	156.0	155.2
Successfully mated (*N*)	23	24	25	25	23	25	25	25
Mating index (%)	96	100	100	100	96	100	100	100
Males without progeny (*N*)	1	1	2	1	NA	NA	NA	NA
Pregnant females (*N*)	NA	NA	NA	NA	23	24	23	24
Fertility index (%)	96	96	92	96	100	96	92	96
Estrous cycle length (days)	NA	NA	NA	NA	5.5	4.6	5.7	5.6
Implantation sites (total/mean)	NA	NA	NA	NA	269/11.7	279/11.6	261/11.3	262/10.9
Postimplantation loss (total/%)	NA	NA	NA	NA	16/5.6	16/5.7	14/5.3	23/9.5

From Schneider et al. (2004c)

NA, not applicable; * $P < 0.05$; ** $P < 0.01$

[a] Doses were 30 mg/kg bw per day for F_0, mating A, and 20 mg/kg bw per day for F_0, mating B, and F_1, mating A.

[b] Doses were 75 mg/kg bw per day for F_0, mating A, and 50 mg/kg bw per day for F_0, mating B, and F_1, mating A.

[c] Two mid-dose and two high-dose males were already infertile at the first mating.

0, respectively). The increased rate of pup deaths was mainly attributable to improper nursing behaviour by four dams that lost all their pups within 2 weeks after birth; a fifth dam that showed normal nursing behaviour also lost all of her pups. Increased pup death resulted in a significant reduction in the viability index (93%) and the lactation index (70%) at the high dose level. No clinical signs were observed in F_{1A} pups up to weaning. Pups showed a significant reduction in mean body weight on postnatal day (PND) 4 and PND 7 and in mean body weight gain on PNDs 1–4 and PNDs 4–7, whereas body weight and body weight gain were similar to those of controls after PND 7. At necropsy, a significant increase in the number of high-dose pups with empty stomachs was noted, resulting from improper nursing behaviour in dams (Table 27).

F_{1B} litter production: At F_{1A} weaning, treatment levels for mid- and high-dose F_0 parental animals were reduced to 20 (from 30) and 50 (from 75) mg/kg bw per day. Parental animals were mated again with the same partners as for the F_{1A} litters. Two mid-dose and two high-dose females died. Poor general state was noted for one mid-dose and seven high-dose females during premating and for three high-dose dams during gestation (Table 26). During lactation, one high-dose female did not nurse her pups properly, and none survived. Another dam showed poor general state, did not clean her pups after birth, did not cut the umbilical cords and did not consume the placentae; consequently, all live pups died on the day of birth. Significantly lower group mean maternal body weights were recorded for high-dose dams at the start of the F_{1B} premating period compared with controls (as a result of the lower maternal body weights during production of the F_{1A} litters) and continued throughout production of the F_{1B} litters, although the corresponding maternal body weight gains during the same period for high-dose females (50 mg/kg bw per day) were comparable with those of controls. Infertility was observed in three mid-dose pairs and three high-dose pairs. However, one mid-dose and one high-dose pair had already proved their fertility, whereas two of the mid- and high-dose pairs were already infertile at the first mating. No conclusive adverse histopathological findings occurred that could account for the observed impaired male fertility. Also, evaluation of sperm parameters (count, morphology, motility) from control and high-dose males revealed no treatment-related effects. No treatment-related necropsy or histopathological findings were observed in F_0 parental animals.

The mean number of delivered F_{1B} pups per dam was not affected at any dose. The number of stillborn pups was significantly increased and the number of liveborn pups significantly decreased at 50 mg/kg bw per day compared with controls (Table 27), whereas the live birth index at the high dose (94%) was slightly outside the historical control range (96–100%). Postnatal survival was also impaired at the high dose level: 14 high-dose F_{1B} pups died and 12 were cannibalized, compared with 1 and 0 for controls, respectively. There were three dams with complete litter losses, and two of these dams showed improper nursing behaviour. All three dams showed poor general state during the first litter, with one continuing to show poor general state during gestation and lactation of the second litter. The viability index (92%) and the lactation index (92%) at the high dose level were both significantly decreased compared with controls (100%), whereas low- and mid-dose values were not affected. No clinical signs and no effects on body weight or body weight gain were observed in F_{1B} pups up to weaning. At necropsy, no treatment-related effects were noted.

F_2 litter production: The reproduction parameters, including evaluation of sperm parameters (count, morphology, motility) and estrous cycle data, of the F_{1B} males and females selected for the production of F_2 litters revealed no treatment-related effects. The fertility indices for F_1 males were comparable with that of controls (96%, 96%, 92% and 96% at 0, 12, 20 and 50 mg/kg bw per day, respectively). Also, F_1 female reproduction and delivery parameters were comparable with those of controls (the fertility index was 100%, 96%, 92% and 96% at 0, 12, 20 and 50 mg/kg bw per day, respectively). All pregnant dams had live F_2 pups in their litters (gestation index of 100% for all groups). No treatment-related changes were noted for F_1 parental clinical signs, food consumption, body weight, necropsy or histopathology. F_2 pup viability, development, mortality, sex ratios, clinical signs, body weights and necropsy findings were not affected by treatment.

Table 27. Summary of selected findings for F_{1A}, F_{1B} and F_2 litters in a two-generation reproductive toxicity study in rats

Parameter	Dose level (mg/kg bw per day)			
	0	12	30/20[a]	75/50[b]
F_{1A} litter				
Females with liveborn (*N*)/gestation index (%)	24/100	25/100	21/100	18/100
Pups delivered, total (*N*)/mean	257/10.7	284/11.4	199/9.5	165/9.2
Pups liveborn (*N*)/live birth index (%)	257/100	279*/98	197/99	163/99
Pups stillborn (*N*)	0	5*	2	2
Pups died/cannibalized (*N*)	1/0	1/2	3/1	43**/7**
Pups surviving days 0–4 (*N*)/viability index (%)	255/99	276/99	194/98	151**/93**
Pups surviving days 4–21 (*N*)/lactation index (%)	188/100	195/100	151/99	90**/70**
Complete litter losses (*N*)	0	0	1	5
Body weight (g), day 1; males/females	6.4/6.2	6.3/5.9	6.5/6.1	6.6/6.3
Body weight (g), day 7; males/females	14.9/14.6	14.9/14.3	14.6/14.2	13.6/12.6**
Body weight (g), day 21; males/females	46.1/45.4	46.0/44.6	46.6/45.6	44.7/43.2
Pups with empty stomach at necropsy (*N*)/litters (*N*)	0	0	1/1	33/9**
F_{1B} litter				
Females with liveborn (*N*)/gestation index (%)	24/96	24/96	21/100	20/95
Pups delivered; total (*N*)/mean	232/9.3	236/9.4	195/9.3	191/9.6
Pups liveborn (*N*)/live birth index (%)	227/98	232/98	194/99	179*/94
Pups stillborn (*N*)	5	4	1	12*
Pups died/cannibalized (*N*)	1/0	1/2	1/2	14**/12**
Pups surviving days 0–4 (*N*)/viability index (%)	226/100	231/100	191/98	164**/92*
Pups surviving days 4–21 (*N*)/lactation index (%)	182/100	172/99	155/100	127**/92*
Complete litter losses (*N*)	0	0	0	3
Body weight (g), day 1; males/females	6.8/6.4	6.6/6.3	6.9/6.7	6.7/6.3
Body weight (g), day 7; males/females	15.6/15.0	15.5/14.7	16.0/15.7	14.9/14.3
Body weight (g), day 21; males/females	48.7/46.7	48.1/45.9	50.2/49.0	46.6/44.8
Pups with empty stomach at necropsy (*N*)/litters (*N*)	1/1	0	0	4/2
F_2 litter				
Females with liveborn (*N*)/gestation index (%)	23/100	24/100	23/100	24/100
Pups delivered; total (*N*)/mean	253/11.0	263/11.0	247/10.7	239/10.0
Pups liveborn (*N*)/live birth index (%)	249/98	262/100	244/99	234/98
Pups stillborn (*N*)	4	1	3	6
Pups died/cannibalized (*N*)	8/7	2/3	1/6	7/4
Pups surviving days 0–4 (*N*)/viability index (%)	235/94	257/98	238/98	226/97
Pups surviving days 4–21 (*N*)/lactation index (%)	175/99	186/100	180/99	172/98
Body weight (g), day 1; males/females	6.4/6.0	6.4/6.1	6.5/6.2	6.5/6.1
Body weight (g), day 7; males/females	15.4/15.0	14.9/144.4	15.1/14.6	14.7/14.0
Body weight (g), day 21; males/females	48.7/47.6	48.8/47.0	48.3/47.1	47.3/45.1
Pups with empty stomach at necropsy (*N*)/litters (*N*)	4/1	0	0	0

From Schneider et al. (2004c)
* $P < 0.05$; ** $P < 0.01$
[a] Doses were 30 mg/kg bw per day for F_{1A} litter and 20 mg/kg bw per day for F_{1B} and F_2 litters.
[b] Doses were 75 mg/kg bw per day for F_{1A} litter and 50 mg/kg bw per day for F_{1B} and F_2 litters.

The NOAEL for parental toxicity was 20 mg/kg bw per day, based on increased incidences of poor general state of health of females at 30 mg/kg bw per day and above. The NOAEL for offspring toxicity was 20 mg/kg bw per day, based on increased incidences of stillborn pups and increased pup mortality at 50 mg/kg bw per day and above. The NOAEL for effects on fertility was 50 mg/kg bw per day, based on a reduction in the male and female fertility indices at 75 mg/kg bw per day (Schneider et al., 2004c).

(b) Developmental toxicity

In a prenatal developmental toxicity study, groups of 25 time-mated female Wistar (CrlGlxBrlHan:WI) rats received metaflumizone (purity 96.9%) by oral gavage at dose levels of 0, 15, 40 and 120 mg/kg bw per day starting on day 6 through day 19 postcoitum (p.c.). Control animals received the vehicle, 0.5% CMC in distilled water, at the same dose volume (10 ml/kg bw) as administered to the treated animals. The number of implantation sites obtained was sufficient (19–25) to allow a meaningful assessment. Clinical parameters, including food consumption, body weight and body weight gains, were recorded, and all animals were assessed in gross pathological examinations, including weight determination of the unopened uterus and the placentae. Reproduction data of the dams—namely, the number of corpora lutea and the number and distribution of implantation sites—were determined. Examinations of the fetuses included assessment of sexes, weights and external findings. Half of the fetuses of each litter were investigated for soft tissue findings, and the other half of the fetuses were examined for skeletal, including cartilage, findings.

There were no maternal deaths or treatment-related clinical signs. At 120 mg/kg bw per day, mean food consumption was statistically significantly reduced (about 9% below the control value on treatment days 10–15 p.c. and about 6% below the control value for the entire treatment period). Also at 120 mg/kg bw per day, a statistically significant decrease in body weight gain (about 54% below control values) was noted on days 6–8 p.c., whereas absolute and corrected body weight gains during the entire treatment period (days 6–19 p.c.) were about 12% and 22% below controls, respectively. No signs of substance-induced maternal toxicity occurred at the low and the middle dose levels (Table 28).

There were no substance-related influences on the gestational parameters up to and including the highest dose level. Conception rate, mean number of corpora lutea, total implantations, resorptions and live fetuses, fetal sex ratio and the values calculated for the preimplantation and the postimplantation losses were unaffected by treatment. Placental and fetal body weights were unaffected. The external, soft tissue and skeletal (including cartilage) examinations of the fetuses revealed no biologically relevant differences between the control and the substance-treated groups.

The NOAEL for maternal toxicity was 40 mg/kg bw per day, based on decreased food consumption and decreased absolute and corrected body weight gains at 120 mg/kg bw per day. The NOAEL for prenatal developmental toxicity was 120 mg/kg bw per day, the highest dose tested (Schneider et al., 2004b).

In a prenatal developmental toxicity study, groups of 25 inseminated female Himalayan (Chbb:HM) rabbits received metaflumizone (purity 96.9%) by oral gavage at dose levels of 0, 30, 100 and 300 mg/kg bw per day starting on day 6 through day 28 post-insemination (p.i.). Control animals received the vehicle, 0.5% CMC in distilled water, at the same dose volume (10 ml/kg bw) as administered to the treated animals. The number of implantation sites obtained was sufficient (19–23) to allow a meaningful assessment. Clinical parameters, including food consumption, body weight and body weight gains, were recorded, and all animals were assessed in gross pathological examinations, including weight determination of the unopened uterus and the placentae. Reproduction data of the does—namely, the number of corpora lutea and the number and distribution of implantation sites—were determined. Examinations of the fetuses included assessment of sexes, weights and external findings. Half of the fetuses of each litter were investigated for soft tissue findings, and the other half of the fetuses were examined for skeletal, including cartilage, findings.

Table 28. Summary of selected findings in a prenatal developmental toxicity study in rats

Parameter	Dose level (mg/kg bw per day)			
	0	15	40	120
Number of mated females on study	25	25	25	25
Number of pregnant females	21	25	23	19
Food consumption (g/animal per day)				
- Days 0–6 p.c.	15.5	15.7	15.4	15.6
- Days 6–8 p.c.	18.0	17.8	17.6	17.4
- Days 8–10 p.c.	18.6	18.8	17.8	17.6
- Days 10–13 p.c.	19.8	19.9	19.2	18.1*
- Days 13–15 p.c.	20.2	20.3	19.5	18.3**
- Days 15–17 p.c.	20.5	20.4	19.9	19.8
- Days 6–19 p.c.	19.8	20.0	19.2	18.6
Body weight (g)				
- Day 0	158.6	163.8	162.4	164.1
- Day 6 p.c.	184.9	190.3	189.3	190.4
- Day 8 p.c.	191.6	195.4	194.7	193.4
- Day 10 p.c.	201.5	206.0	203.1	202.4
- Day 13 p.c.	212.2	216.8	213.7	211.3
- Day 15 p.c.	222.0	226.3	222.4	219.8
- Day 19 p.c.	254.2	260.7	253.5	251.4
Body weight gain (g)				
- Days 0–6 p.c.	26.3	26.5	26.8	26.2
- Days 6–8 p.c.	6.7	5.1	5.5	3.1**
- Days 8–10 p.c.	9.9	10.5	8.4	8.9
- Days 10–13 p.c.	10.7	10.8	10.6	8.9
- Days 13–15 p.c.	9.8	9.5	8.7	8.5
- Days 6–19 p.c.	69.3	70.4	64.2	61.0
Corrected body weight gain (g), days 6–19 p.c.	33.9	31.7	29.3	26.3*
Implantation index (%)	93.8	88.9	89.6	87.1
Viability index (%)	93.9	96.1	91.4	90.3

From Schneider et al. (2004b)
* $P < 0.05$; ** $P < 0.01$

At 300 mg/kg bw per day, maternal toxicity was evident, as indicated by adverse clinical symptoms in four rabbits, such as lateral position, ataxia, poor general state, blood in bedding and/or no defecation (from day 22 p.i. onwards). Two of these females aborted prior to being sacrificed early (on days 22 and 28 p.i.), and a third female at 300 mg/kg bw per day was sacrificed moribund (on day 28 p.i.). No treatment-related effects were indicated for food consumption, body weight/body weight gain or necropsy findings. No signs of substance-induced maternal toxicity occurred at the low or the middle dose levels (Table 29).

There were no substance-related influences on the gestational parameters up to and including the highest dose level. Conception rate, mean number of corpora lutea, total implantations, resorptions and live fetuses, fetal sex ratio and the values calculated for the preimplantation and the postimplantation losses were unaffected by treatment.

Table 29. Summary of selected findings in a prenatal developmental toxicity study in rabbits

Parameter	Dose level (mg/kg bw per day)				Historical control
	0	30	100	300	
Mated females on study (*N*)	25	25	25	25	
Pregnant females (*N*)	21	23	19	23	
Animals with clinical signs (*N*)	0	0	0	4	
Abortions (*N*)	1	0	0	2	
Dams with viable fetuses (*N*)	20	23	19	20	
Corpora lutea (*N*)	7.6	7.4	8.7	7.8	
Implantation sites (*N*)	6.4	6.3	7.8	6.9	
Preimplantation loss (%)	15.6	15.0	10.3	11.4	
Postimplantation loss (%)	8.4	10.2	11.9	6.1	
Early resorptions (*N*)	0.4	0.3	0.7	0.3	
Late resorptions (*N*)	0.0	0.4*	0.2	0.1	
Live fetuses per dam (*N*)	6.1	5.6	6.9	6.5	
Total litter weights (g)	214.2	209.5	232.1	212.4	
Fetal body weights, M + F combined (g)	36.3	38.1	34.3	33.6	
Runts (%)[a]	3.3	1.6	5.3	13.8	
Absent subclavian artery					
- Fetal incidence (*N*)	0/121	1/129	1/131	3/131	3/805
- Fetal incidence (%)	0	0.8	0.8	2.3	0.4 (0–2.1)[b]
- Litter incidence (*N*)	0/20	1/23	1/19	3/20	3/118
- Litter incidence (%)	0	4.3	5.3	15.0	2.5 (0–13.6)[b]
- Affected fetuses per litter (%)	0	0.6	0.7	3.1*	0.4 (0–1.9)
Incomplete ossification of sternebra: unchanged cartilage					
- Fetal incidence (*N*)	42/121	45/129	66/131	60/131	197/805
- Fetal incidence (%)	35	35	50	46	24.5 (17.1–29.5)
- Litter incidence (*N*)	15/20	18/23	18/19	18/20	92/118
- Litter incidence (%)	75	78	95	90	78.0 (70.8–87.5)
- Affected fetuses per litter (%)	29.7	32.3	47.5*	46.5**	24.2 (16.6–31.6)

From Schneider et al. (2004a)
* $P < 0.05$; ** $P < 0.01$
[a] Runts are defined as fetuses with a body weight of 25% less than the mean weight of the concurrent control.
[b] Upper range incidences in controls from another study performed at the same laboratory: fetal incidence = 3/146 (2.1%), litter incidence = 3/22 (13.6%).

Mean fetal weights were 5.5% and 7.4% lower than those of controls at 100 and 300 mg/kg bw per day, respectively, and the percentage of stunted fetuses/runts was increased at the high dose level (Table 29). No treatment-related fetal external, visceral or skeletal malformations were indicated. The incidence of absent subclavian artery was statistically significantly increased at 300 mg/kg bw per day if expressed on a fetus per litter rate (3.1%); however, this finding occurred at a similar fetal and litter incidence in the historical controls and was therefore not considered to be related to treatment.

The incidence of incomplete ossification of sternebrae (if expressed on a fetus per litter basis) was statistically significantly increased at 100 and 300 mg/kg bw per day and also exceeded the historical control range. The increased occurrence of this variation at the high dose (which reflects a transient and

reversible delay in ossification) was assessed as a treatment-related finding, which is in line with the lower fetal body weights and the increased rate of runts in this group. At the middle dose, in the absence of a dose–response relationship, the increased incidence of this finding was not considered to be toxicologically significant, as the mean number of live fetuses per dam in this group was clearly above the concurrent control value, and the mean fetal body weight was slightly below the concurrent control value.

The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on clinical signs of toxicity (lateral position, ataxia, poor general state) and abortions at 300 mg/kg bw per day. The NOAEL for prenatal developmental toxicity was 100 mg/kg bw per day, based on an increased number of runts/stunted fetuses, decreased fetal body weights and an increased rate of incomplete ossification of sternebrae at 300 mg/kg bw per day (Schneider et al., 2004a; Hellwig, 2005).

2.6 Special studies

(a) Neurotoxicity

In an acute neurotoxicity study, groups of 10 male and 10 female Wistar (CrlGlxBrlHan:WI) rats received metaflumizone (purity 95.8%) as an aqueous suspension by oral gavage at doses of 0, 125, 500 and 2000 mg/kg bw. Control animals received the vehicle, 0.5% CMC in distilled water, at the same dose volume (10 ml/kg bw) as administered to the treated animals. The animals were examined for signs of toxicity or mortality at least once a day. Body weight was determined on day −7 (prior to dosing), day 0 (test substance administration), day 1, day 7 and day 14. FOB and motor activity measurements were carried out in all animals on day −7, on day 1 (25 h after treatment) and on days 7 and 14. Five animals of each sex per dose were fixed by in situ perfusion and subjected to neuropathological examinations. The remaining animals were sacrificed under carbon dioxide anaesthesia without further examinations.

No animals died during the study period. No treatment-related clinical signs were revealed in the FOB or motor activity measurements at dose levels up to 2000 mg/kg bw. No treatment-related lesions were observed in the neuropathological examinations.

Under the conditions of this study, the NOAEL for acute neurotoxicity in male and female rats was 2000 mg/kg bw, the highest dose tested (Kaspers et al., 2003a).

In a subchronic neurotoxicity study, groups of 10 male and 10 female Wistar (CrlGlxBrlHan:WI) rats received metaflumizone (purity 95.8%) as an aqueous suspension by oral gavage at doses of 0, 12, 36, 150 (both sexes) and 300 (males only) mg/kg bw per day for 3 months. Control animals received the vehicle, 0.5% CMC in distilled water, at the same dose volume (10 ml/kg bw) as administered to the treated animals. Food consumption was determined once a week. Body weight was determined once a week and on the days when FOBs were performed. The animals were examined daily before and after treatment. FOB and motor activity measurements were carried out on days −7, 1, 22, 50 and 85. Ophthalmological examinations were carried out in all animals before treatment and in control as well as high-dose animals at the end of the administration period. Five animals of each sex per dose were fixed by in situ perfusion and subjected to neuropathological examinations.

One male and one female in the 150 mg/kg bw per day group died on days 59 and 16, respectively. The mean body weights, body weight gain and food consumption of the females in the 150 mg/kg bw per day group and males in the 300 mg/kg bw per day group were less than those of the control animals ($P < 0.01$ or 0.05). These animals also demonstrated increased incidences of discoloured faeces, piloerection or hypothermia. Piloerection was also noted in the FOB as an effect for animals in these groups at various time points during the study. In motor activity assessment, lower numbers of beam interruptions were noted for females in the 150 mg/kg bw per day group and males in the 300 mg/kg bw per day group at various time points during the study. However, no consistent pattern of effect was elicited by the treatment. No treatment-related lesions were noted in the histopathological examination.

Under the conditions of this study, the NOAELs for neurotoxicity were 300 mg/kg bw per day in males and 150 mg/kg bw per day in females (the highest doses tested, respectively). The NOAEL for general toxicity was 36 mg/kg bw per day, based on clinical findings of poor general state, including mortality and impairment of food consumption and body weight at 150 mg/kg bw per day (Kaspers et al., 2003b; Kaspers, 2005).

In a range-finding developmental neurotoxicity study, groups of 20 presumed pregnant female Wistar (CrlGlxBrlHan:WI) rats received metaflumizone (purity 96.9%) by oral gavage at dose levels of 0, 50, 80 and 120 mg/kg bw per day. Each dose group was subdivided into two cohorts (A and B) consisting of 10 animals each. The animals of cohort A were treated with the test substance on day 6 p.c. through day 10 p.p. The animals of cohort B were treated with the test substance on day 6 p.c. through day 21 p.p. A standard dose volume of 10 ml/kg bw was used for all animals. The control group, consisting of 20 presumed pregnant female rats, was also subdivided into cohorts A and B and was dosed with the vehicle only (0.5% CMC in distilled water). The dams were allowed to litter and rear their pups until day 4 or 21 p.p. After the pups were weaned (day 21 p.p.), the dams were sacrificed and assessed by gross pathology. The state of health of the dams and pups was checked each day. Food consumption of the dams was determined regularly during gestation (days 0–6, 6–13 and 13–20) and lactation periods (days 0–1, 1–7, 7–14 and 14–21). Body weights of the dams were determined regularly during gestation (days 0, 6, 13 and 20) and lactation periods (days 0, 1, 7, 14 and 21). For the determination of test substance residues in milk, all dams with litters in cohort A were milked on lactation day 10 (last day of dosing) as well as lactation days 11, 14, 17 and 21 (after cessation of dosing). All dams with litters in cohort B were milked on lactation days 10, 14 and 21 (continuous dosing). The pups were sexed and examined for macroscopically evident changes on the day of birth. The live pups were examined daily for clinical symptoms (including gross morphological findings) during the clinical inspection of the dams. All pups were weighed on the day after birth and on days 4, 7, 14 and 21 p.p., and their viability was recorded. Blood samples were taken from individual pups of each test group and each cohort on days 0, 4, 10 and 21 p.p. for the determination of the test substance concentration in blood plasma. All pups, with the exception of the pups for blood sampling on days 0, 4 and 10 p.p., were examined macroscopically at necropsy. All pups without any notable findings or abnormalities were discarded after their macroscopic evaluation.

No dams died during the study period. At 120 mg/kg bw per day, poor general state and piloerection during major parts of gestation and lactation were observed in one dam of cohort A, whereas insufficient nursing and subsequent litter loss were seen on day 2 p.p. in one dam of cohort B and on day 5 p.p. in one dam of cohort A (Tables 30 and 31). No adverse effects were noted in dams at a dose level of 80 mg/kg bw per day and below. No treatment-related findings were noted in pups at dose levels up to 120 mg/kg bw per day, the highest dose level tested.

Under the conditions of this range-finding study, the NOAEL for general toxicity and reproductive performance was 80 mg/kg bw per day, based on clinical signs of poor general state and litter loss in individual dams at 120 mg/kg bw per day (Schneider, 2005).

As a part of the range-finding developmental neurotoxicity study described above, residue levels were monitored in milk of the dams and plasma of the newborn rats. Owing to the small sample size, some modifications were applied to the method, which was revalidated with a higher limit of quantification (LOQ) before starting analysis. After addition of methanol to a milk sample (plasma samples were already diluted with methanol after sampling), the sample was mixed and centrifuged. For cleanup, a liquid/liquid partition step against dichloromethane was performed. The determination of the test substance was performed by means of HPLC with tandem mass spectrometry (MS/MS). The theoretical LOQ for milk was 0.01 mg/kg for total metaflumizone or 0.005 mg/kg for each isomer. Owing to small sample size and expected higher residues, the meth-

Table 30. Summary of selected findings in a range-finding developmental neurotoxicity study in rats: cohort A

Parameter	Dose level (mg/kg bw per day)			
	0	50	80	120
Females on study (*N*)	10	10	10	10
Clinical signs: poor general state; piloerection (*N*)	0	0	0	0
Pups not properly nursed: no more pups alive (*N*)	0	0	0	1
Mated females (*N*)	10	10	10	10
Pregnant females (*N*)	10	9	10	10
Implantation sites (total) (*N*)	96	87	88	90
Postimplantation loss (mean *N*)	0.3	0.0	0.1	0.1
% postimplantation loss	3.8	0.0	1.0	0.8
Females with liveborn (*N*)	10	9	10	10
Gestation index (%)	100	100	100	100
Pups delivered (total) (*N*)	93	87	87	89
- Liveborn (*N*)	92	87	86	88
- Stillborn (*N*)	1	0	1	1
Pups died (*N*)	0	0	2	5*
Pups sacrificed moribund (*N*)	0	0	0	0
Pups cannibalized (*N*)	1	0	0	4
Pups accidental death (*N*)	0	0	0	0
Pups culled day 4 (*N*)	14	11	6	8

From Schneider (2005)
* $P < 0.05$

od was validated before starting the sample analysis and by concurrent recoveries obtained from spiked samples in the range of 0.05–10.0 mg/kg for each isomer. Mean recoveries were 87–96% and 90–105% for the *E* isomer and 84–92% and 88–101% for the *Z* isomer in milk and plasma, respectively.

Residues in milk were found in all dosed groups; controls were free of residues. Although there was considerable variation among individuals within a dose group, the average residues found in milk of rats showed an almost linear dose relationship at the beginning of the sampling period (between 5.9 and 14.6 mg/kg in cohort A and between 5.2 and 11.4 mg/kg in cohort B). After the end of dosing (cohort A), residues in milk declined rapidly to levels of 0.5–0.8 mg/kg at the end of the sampling period. When the administration of the test item continued throughout the study (cohort B), only a moderate decline to constant residue levels in milk was observed. The group average residue values are summarized in Table 32.

Based on the results of this study, an estimation of the exposure level of the pups via milk depending on the dose administered to the dams can be made. Average plasma levels of the test compound in pups were much lower than the levels in milk. They increased from low levels after birth (0.26–0.50 mg/kg) to peak levels of 4.0 mg/kg (cohort B). In all cases, plasma levels decreased at the end of the study period (Tilting & Mackenroth, 2005).

(b) Studies with M320I02 (Z isomer of metaflumizone)

In an acute oral toxicity study, groups of three male and three female fasted Sprague-Dawley (Crl:CD) rats received M320I02 (purity 96.9%) by oral gavage at a single dose of 5000 mg/kg bw in

Table 31. Summary of selected findings in a range-finding developmental neurotoxicity study in rats: cohort B

Parameter	Dose level (mg/kg bw per day)			
	0	50	80	120
Females on study (*N*)	10	10	10	10
Clinical signs: poor general state; piloerection (*N*)	0	0	0	1
Pups not properly nursed: no more pups alive (*N*)	0	0	0	1
Mated females (*N*)	10	10	10	10
Pregnant females (*N*)	9	10	10	9
Implantation sites (total) (*N*)	87	91	86	81
Postimplantation loss (mean *N*)	0.2	0.2	0.0	0.3
% postimplantation loss	2.5	2.4	0.0	3.7
Females with liveborn (*N*)	9	10	10	9
Gestation index (%)	100	100	100	100
Pups delivered (total) (*N*)	85	89	86	78
- Liveborn (*N*)	83	88	86	78
- Stillborn (*N*)	2	1	0	0
Pups died (*N*)	0	0	0	5*
Pups sacrificed moribund (*N*)	0	0	0	0
Pups cannibalized (*N*)	0	1	0	5*
Pups accidental death (*N*)	0	0	0	0
Pups culled day 4 (*N*)	9	8	6	6

From Schneider (2005)
* $P < 0.05$

0.5% aqueous CMC. The administration volume was 20 ml/kg bw. The observation period lasted for at least 14 days. No mortality occurred. Clinical signs and findings comprised impaired general state, dyspnoea and piloerection. Findings were observed from hour 4 through hour 5 after administration. The mean body weights of both sexes increased throughout the study period. No macroscopic pathological abnormalities were noted at the end of the observation period. Under the conditions of this study, the LD_{50} of the test substance after a single oral administration was found to be greater than 5000 mg/kg bw for male and female rats (Gamer & Leibold, 2002b).

In a subchronic oral toxicity study, groups of 10 male and 10 female Sprague-Dawley CD (Crl:CD (SD) IGS BR) rats received M320I02 (purity 99.7%) by oral gavage at dose levels of 0, 100, 300 and 1000 mg/kg bw per day for a period of 13 weeks. Control animals received the vehicle, 0.5% CMC in distilled water, at the same dose volume (10 ml/kg bw) as administered to the treated animals. Food consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. An FOB and measurement of motor activity were carried out at the end of the administration period. Ophthalmological examinations were conducted on all animals before treatment and on control and high-dose animals at the end of the administration period. Clinicochemical and haematological examinations and urinalyses were performed towards the end of the administration period. Finally, all animals were assessed by gross pathology, followed by histopathological examinations.

Table 32. Mean test substance concentration in milk and plasma samples from a range-finding developmental neurotoxicity study in rats

Parameter	Test substance concentration (mg/kg)							
	Cohort A (substance application until lactation day 10)				Cohort B (substance application until lactation day 21)			
	Dose level (mg/kg bw per day)							
	0	50	80	120	0	50	80	120
Milk (dams)								
- Lactation day 10	<0.1	5.86	9.00	14.63	<0.1	5.15	9.44	11.41
- Lactation day 11	<0.1	5.63	8.26	15.18	—	—	—	—
- Lactation day 14	<0.1	1.67	2.34	3.54	<0.1	3.63	5.55	8.26
- Lactation day 17	<0.1	0.653	1.13	1.49	—	—	—	—
- Lactation day 21	<0.1	0.448	0.606	0.791	<0.1	3.55	5.29	8.21
Plasma (pups)								
- Lactation day 0	<0.1	0.28	0.38	0.50	<0.1	0.26	0.29	0.38
- Lactation day 4	<0.1	1.79	2.00	2.86	<0.1	1.91	4.00	2.79
- Lactation day 10	<0.1	1.35	1.69	2.93	<0.1	0.97	1.63	1.91
- Lactation day 21	<0.1	0.34	0.42	0.66	<0.1	0.66	1.03	1.20

From Tilting & Mackenroth (2005)

One female of the high dose group was found dead on day 77, and one female of the middle dose group was sacrificed moribund on day 77. A treatment relationship of these two deaths is considered likely, as both animals demonstrated clinical signs of toxicity, including abdominal position, ataxia, splayed limbs and reduced general condition. These findings were also observed to a different extent in two additional female animals in the high dose group and in one additional female animal in the middle dose group. Additionally, smeared anogenital region, obviously connected with the reduced general condition, was observed in the female animals of the middle dose group that exhibited clinical signs of toxicity. Reduced food consumption was seen throughout the study in females in the middle and high dose groups. Terminal body weight was decreased in high-dose females (−9.4%) as well as in mid-dose females (−13%). Body weight gain was also reduced.

No treatment-related effect was noted in the ophthalmological, haematological, clinical chemistry or urinalysis examinations. The FOB did not reveal any treatment-related effects. The motor activity of both sexes in the 1000 mg/kg bw per day group and of the females in the 300 mg/kg bw per day group was less than that of the controls. In the necropsy examination, the mean relative liver weights of the females in all of the treatment groups were greater than that of the controls. However, no histopathological effects were associated with this increased weight. The mean relative adrenal gland weights of the females in the 300 and 1000 mg/kg bw per day groups were greater than that of the controls. In the histopathological examination, an increased incidence of centrilobular hypertrophy (grade 1) was noted in the livers of the treated males. For the females, the 1000 mg/kg bw per day group demonstrated decreased cellularity in the spleen (grade 2 or 4), and lymphocyte necrosis (grade 1 or 2) was noted in the mesenteric lymph nodes in the 300 and 1000 mg/kg bw per day groups. Also, the females in the 300 and 1000 mg/kg bw per day groups exhibited a greater severity of vacuolation in the adrenal gland (Table 33).

The NOAEL was 100 mg/kg bw per day, based on reduced body weight/body weight gain, reduced motor activity, increased incidences of lymphocyte necrosis in mesenteric lymph nodes and

Table 33. Summary of selected findings in a 13-week toxicity study in rats with M320I02

Parameter	Males				Females			
	Dose level (mg/kg bw per day)							
	0	100	300	1000	0	100	300	1000
Body weight, terminal (g)	465	488	481	478	277	274	241*	251
Motor activity, day 85/87[a]	225	220	217	181**	391	301	288*	222**
Absolute liver weight (g)	11.31	12.47	11.88	12.25	7.08	7.64	6.79	6.78
Relative liver weight (%)	2.43	2.55	2.47	2.56	2.55	2.81*	2.82*	2.71**
Absolute adrenal weight (mg)	60.3	65.5	64.1	65.8	85.5	91.3	87.1	93.9
Relative adrenal weight (%)	0.013	0.014	0.013	0.014	0.031	0.034	0.036*	0.038*
Hepatocytes; centrilobular hypertrophy (grade 1)[b]	0	1	2	4	0	0	0	0
Spleen, cellularity decrease[b]	0	0	0	0	0	0	0	2
Mesenteric lymph nodes; lymphocyte necrosis[b]	0	0	0	0	0	0	1	3
Adrenal cortex; vacuolation[b]	10	10	10	10	10	9	10	10
- Grade 2	0	4	1	0	0	0	0	0
- Grade 3	10	6	9	10	9	8	2	2
- Grade 4	0	0	0	0	1	1	8	7
- Grade 5	0	0	0	0	0	0	0	1

From Kaspers et al. (2004c)
* $P < 0.05$; ** $P < 0.01$
[a] Sum of beam interruptions.
[b] Number of animals exhibiting finding.

increased severity of adrenal cortex vacuolation in female rats at 300 mg/kg bw per day and above (Kaspers et al., 2004c).

In a reverse mutation assay, *S. typhimurium* (strains TA98, TA100, TA1535 and TA1537) and *E. coli* (strain WP2 *uvr*A) were exposed to M320I02 (purity 96.9%) using DMSO as solvent in the presence and absence of S9 activation in two independent sets of experiments. Triplicate plates were used per dose and per condition. Vehicle and positive controls were included in each experiment. In the plate incorporation assay, concentrations of 0, 20, 100, 500, 2500 and 5000 µg/plate for 48–72 h were used with and without metabolic activation. The preincubation assay was performed at concentrations of 0, 4, 20, 100, 500 and 2500 µg/plate (pretreatment for 20 min in flasks, followed by plating and exposure for 48–72 h). No bacteriotoxicity was observed in the plate incorporation assay, whereas in the preincubation assay, a slight decrease in the number of revertants was occasionally observed, depending on the strain and test conditions, at concentrations of 100 µg/plate or above. Precipitation of the test substance was noted at concentrations of 100 µg/plate and above.

No increase in the number of revertant colonies was noted in any of the strains tested in either the plate incorporation or the preincubation assay in the presence or absence of metabolic activation. The positive controls induced the appropriate responses in the corresponding strains, thus demonstrating the sensitivity of the test system. Under the experimental conditions of the study, the test substance M320I02 is not mutagenic in the *S. typhimurium*/*E. coli* reverse mutation assay (Engelhardt & Leibold, 2002a; Engelhardt, 2005).

Table 34. Summary of selected findings in a 13-week toxicity study in rats

Parameter	Males				Females			
	Dose level (mg/kg bw per day)							
	0	50	200	1000	0	50	200	1000
Body weight, terminal (g)	500	536	487	469	277	281	280	267
Absolute liver weight (g)	13.35	13.86	12.63	12.76	6.93	7.49	7.77	7.66
Relative liver weight (%)	2.67	2.59	2.59	2.71	2.51	2.66	2.77*	2.87**
Absolute adrenal weight (mg)	65.7	66.3	65.7	66.1	70.1	75.9	86.2**	81.6**
Relative adrenal weight (%)	0.013	0.012	0.014	0.014	0.025	0.027	0.031**	0.031*
Hepatocytes; centrilobular hypertrophy (*N*)	3	0	3	9	1	0	0	6
Thyroid; follicular hypertrophy (*N*)	2[A]	6	7	9	1	1	1	1

From Kaspers et al. (2004d)
* $P < 0.05$; ** $P < 0.01$
[a] Incidence was 7/10 in controls from a parallel 3-month study.

(c) Studies with Reg. No. 4984051 (M320I23) (plant and soil metabolite of metaflumizone)

In an acute oral toxicity study, Reg. No. 4984051 (purity 98.7%) was administered in a sequential manner to two groups of three female fasted Wistar (CrlGlxBrlHan:WI) rats by oral gavage at a single dose of 2000 mg/kg bw in 0.5% aqueous CMC. The administration volume was 10 ml/kg bw. The observation period lasted for at least 14 days. No mortality occurred, and no clinical signs or findings were observed. The mean body weights increased throughout the study period. No macroscopic pathological abnormalities were noted at the end of the observation period. Under the conditions of this study, the LD_{50} of the test substance after a single oral administration was found to be greater than 2000 mg/kg bw for female rats (Gamer & Leibold, 2003; Gamer, 2004).

In a subchronic oral toxicity study, groups of 10 male and 10 female Sprague-Dawley (Crl:CD (SD) IGS BR) rats received Reg. No. 4984051 (purity 98.7%) by oral gavage at dose levels of 0, 50, 200 and 1000 mg/kg bw per day for a period of 13 weeks. Control animals received the vehicle, 0.5% CMC in distilled water, at the same dose volume (10 ml/kg bw) as administered to the treated animals. Food consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. An FOB and measurement of motor activity were carried out at the end of the administration period. Ophthalmological examinations were conducted in all animals before treatment and in control and high-dose animals at the end of the administration period. Clinicochemical and haematological examinations and urinalyses were performed towards the end of the administration period. Finally, all animals were assessed by gross pathology, followed by histopathological examinations.

All male animals and four females of the high dose group showed slight salivation on several days from day 23 until the end of the study, whereas one male of the middle dose group showed slight salivation on days 88 and 89. This finding was observed only within a few minutes after the daily test substance administration and was considered to be due to the physical properties of the test substance rather than indicating systemic toxicity. No treatment-related findings were seen with regard to food and water consumption, body weight and food conversion efficiency. Also, no treatment-related findings were observed in the FOB, motor activity, ophthalmology, haematology, clinical chemistry or urinalysis examinations.

In the necropsy examination, the mean absolute adrenal weights and mean relative liver and adrenal weights of the 200 and 1000 mg/kg bw per day females were greater than those of the controls. An increased incidence of central hypertrophy was noted in the livers of both sexes in the 1000 mg/kg bw per day group. An increased incidence of follicular cell hypertrophy was noted for the males in all of the treatment groups. However, upon closer examination, it was concluded that the incidence reported for the control group animals was low in comparison with the results noted in a parallel 3-month study (Table 34).

The NOAEL was 200 mg/kg bw per day, based on increased salivation in both sexes and increased relative liver weights and centrilobular hypertrophy of hepatocytes in females at 1000 mg/kg bw per day, the highest dose tested (Kaspers et al., 2004d).

In a reverse mutation assay, *S. typhimurium* (strains TA98, TA100, TA1535 and TA1537) and *E. coli* (strain WP2 *uvr*A) were exposed to Reg. No. 4984051 (purity 98.7%) in the presence and absence of S9 activation in two independent sets of experiments. Triplicate plates were used per dose and per condition. Vehicle (DMSO) and positive controls were included in each experiment. In the plate incorporation assay, concentrations of 0, 20, 100, 500, 2500 and 5000 µg/plate for 48–72 h were used with and without metabolic activation. A slight decrease in the number of revertant colonies was observed at concentrations of 500 µg/plate and above, depending on the strain and test. Accordingly, concentrations of 62.5, 125, 250, 500 and 1500 µg/plate were used in the preincubation assay (pretreatment for 20 min in flasks, followed by plating and exposure for 48–72 h). Precipitation of the test substance was noted at concentrations of 250 µg/plate and above. No increase in the number of revertant colonies was noted in either the plate incorporation or the preincubation assay in any of the strains tested in the presence or absence of metabolic activation. The positive controls induced the appropriate responses in the corresponding strains, thus demonstrating the sensitivity of the test system. Under the experimental conditions of the study, the test substance Reg. No. 4984051 is not mutagenic in the *S. typhimurium*/*E. coli* reverse mutation assay (Engelhardt & Leibold, 2003b; Engelhardt, 2004a).

Reg. No. 4984051 (purity 98.7%) was tested in vitro for the ability to induce forward mutations in mammalian cells by assessing the mutation of the *HPRT* locus in Chinese hamster V79 cells (substrain K1) in vitro. Four independent sets of experiments were conducted in the presence (experiments 1–3) and absence (experiments 1–4) of metabolic activation. Based on the results of a preliminary cytotoxicity assay, concentrations of 0.125–6.0 µg/ml and 1.25–100 µg/ml were used in the absence or presence of S9 mix, respectively. EMS and MCA served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 h in the experiments both without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated for about 1 week for expression of mutant cells. This was followed by a 1-week incubation of cells in selection medium containing 6-thioguanine. A reduction in the cloning efficiency was noted at 2.5 µg/ml and higher in the absence of S9 and at 20 µg/ml and higher with S9 mix, after the 4 h exposure period. At the end of the expression period, cytotoxicity was noted at 3 µg/ml and higher without S9 mix and at 30 µg/ml and higher with it. There was no increase in the number of mutant colonies either without S9 mix or after the addition of a metabolizing system in several experiments performed independently of each other. The mutant frequencies at any dose were close to the range of that of the concurrent negative control values and within the range of the historical control data. The increase in the frequencies of mutant colonies induced by the positive control agents EMS and MCA clearly demonstrated the sensitivity of the test method and of the metabolic activity of the S9 mix employed. Based on the results of the study, it is concluded that under the conditions of the test, Reg. No. 4984051 does not induce forward mutations in mammalian cells in vitro (Engelhardt, 2004g; Engelhardt & Leibold, 2004c).

Reg. No. 4984051 (purity 98.7%) was tested in vitro for the ability to induce chromosomal and numerical aberrations in Chinese hamster V79 cells in two independent experiments in the presence and absence of metabolic activation. Based on the results of preliminary cytotoxicity assays, concentrations of 0.25–1.0 µg/ml and 1.0–12.5 µg/ml were tested without and with metabolic activation, respectively, with 4 h treatment followed by 14 h of recovery. Vehicle (DMSO) and positive controls (CPP and EMS for the experiments with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Prior to cell harvest, addition of colcemid arrested cells in the metaphase. After slide preparation and staining of the cells, 200 well-spread metaphases per dose and treatment condition were analysed for chromosomal aberrations. In the experiment without metabolic activation, no increase in the number of aberrant cells was observed up to 1 µg/ml. Higher concentrations could not be evaluated, as total cell death was observed at concentrations of 5 µg/ml and above. The first experiment with metabolic activation revealed a statistically significant increase in the number of aberrant cells at 10 µg/ml. At the next higher concentration of 20 µg/ml, no evaluation was possible as a result of complete cell death. In a second experiment with metabolic activation, a statistically significant increase in aberrant cells was observed at concentrations of 7.5, 10.0 and 12.5 µg/ml. Based on the results of this study, Reg. No. 4984051 is considered to have clastogenic potential in the presence of metabolic activation under the given experimental conditions of this in vitro study in Chinese hamster V79 cells (Engelhardt, 2004f; Engelhardt & Leibold, 2004a).

In a micronucleus test, groups of five male Crl:NMRI mice received two oral gavage doses (24 h apart) of Reg. No. 4984051 (purity 98.7%) at 0 (DMSO and olive oil), 500, 1000 and 2000 mg/kg bw in a volume of 10 ml/kg bw. The vehicle served as negative control, and CPP (20 mg/kg bw) and vincristine sulfate (0.15 mg/kg bw) as positive controls. The animals were sacrificed 24 h after the last administration, the bone marrow of the two femora was prepared and 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The number of normochromatic erythrocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes was also recorded. No clinical signs of toxicity were observed in any group after treatment. Administration of Reg. No. 4984051 did not lead to any increase in the number of micronucleated polychromatic erythrocytes. No effects on the ratio of normochromatic to polychromatic erythrocytes were observed in any of the test article–treated or positive control groups. The positive control chemicals led to the expected increase in the rate of polychromatic erythrocytes containing small (CPP) or small and large (vincristine sulfate) micronuclei, thus demonstrating the sensitivity of the test system (Engelhardt & Leibold, 2004b).

(d) Studies with Reg. No. 43455 (M320I29) (soil metabolite of metaflumizone)

In a reverse mutation assay, *S. typhimurium* (strains TA98, TA100, TA1535 and TA1537) and *E. coli* (strain WP2 *uvr*A) were exposed to Reg. No. 43455 (purity 99.9%) in the presence and absence of S9 activation in three independent sets of experiments (two standard plate incorporation tests and one preincubation test). Triplicate plates were used per dose and per condition. Vehicle (DMSO) and positive controls were included in each experiment. In the plate incorporation assay, Reg. No. 43455 was tested up to the limit concentration of 5000 µg/plate. In the first assay, concentrations of 20, 100, 500, 2500 and 5000 µg/plate were used. The second plate incorporation test and the preincubation test used concentrations of 250, 500, 1000, 1500 and 2000 µg/plate. All tests were run with and without metabolic activation. A bacteriotoxic effect was observed, depending on the strain and test conditions, from about 1500–2000 µg/plate onwards. No precipitation of the test substance was noted. No increase in the number of revertant colonies was noted in any of the strains tested in the presence or absence of metabolic activation. The positive controls induced the appropriate responses in the corresponding strains, thus demonstrating the sensitivity of the test system. Under the experimental conditions of the study, the test substance Reg. No. 43455 is not mutagenic in the *S. typhimurium*/*E. coli* reverse mutation assay (Engelhardt & Leibold, 2004d).

Reg. No. 43455 (purity 99.9%) was tested in vitro for the ability to induce forward mutations in mammalian cells by assessing the mutation of the *HPRT* locus in Chinese hamster V79 cells (substrain K1) in vitro. Two independent sets of experiments were conducted in the presence and absence of metabolic activation. A third independent experiment was conducted in the presence of metabolic activation. Based on the results of a preliminary cytotoxicity assay, concentrations of 125–2000 µg/ml and 125–1500 µg/ml were used in the first experiment with and without metabolic activation, respectively. In the confirmatory experiments, concentrations of 500–1750 µg/ml were used without metabolic activation, whereas concentrations of 125–1250 µg/ml and 1000–2000 µg/ml were used with metabolic activation. EMS and MCA served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 h in all the experiments. After this, the incubation media were replaced by culture medium, and the cells were incubated for about 1 week for expression of mutant cells. This was followed by a 1-week incubation of cells in selection medium containing 6-thioguanine. No increase in the mutant frequency was observed in either the first or the confirmatory studies. In contrast to this, the positive control substances EMS and MCA resulted in a marked increase in mutant frequency. Based on the results of the study, it is concluded that, under the conditions of the test, Reg. No. 43455 does not induce forward mutations in mammalian cells in vitro (Engelhardt & Leibold, 2004e).

Reg. No. 43455 (purity 99.9%) was tested in vitro for the ability to induce chromosomal and numerical aberrations in Chinese hamster V79 cells in two independent experiments in the presence and absence of metabolic activation at concentrations up to 2000 µg/ml, representing the limit concentration of 10 mmol/l. Based on the results of preliminary cytotoxicity assays, concentrations of 125–2000 µg/ml were tested for clastogenic effects with and without metabolic activation in the original experiment with 4 h of treatment followed by 14 h of recovery. The confirmatory experiment was divided into three parts. In the parts without metabolic activation, V79 cells were continuously treated for 18 h and harvested after 18 h (125–2000 µg/ml) or 28 h (500–2000 µg/ml). In the part with metabolic activation, the cells were treated for 4 h and harvested after 28 h (125–2000 µg/ml). Vehicle (DMSO) and positive controls (CPP and EMS for the experiments with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. About 2–3 h prior to cell harvest, addition of colcemid arrested cells in the metaphase. After slide preparation and staining of the cells, 200 well-spread metaphases per dose and treatment condition were analysed for chromosomal aberrations. No increase in the incidence of aberrant cells was observed in the original or confirmatory experiments. In contrast, the positive controls EMS and CPP resulted in a statistically significant increase in the number of aberrant cells, thus demonstrating the sensitivity of the test system. Under the experimental conditions of this in vitro assay in V79 cells, Reg. No. 43455 is not considered to be a clastogenic or an aneugenic agent (Engelhardt & Leibold, 2005).

3. Observations in humans

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisonings with metaflumizone.

Comments

Biochemical aspects

In rats given [^{14}C]benzonitrile ring (B)–labelled or [^{14}C]trifluoromethoxyphenyl ring (T)–labelled metaflumizone orally by gavage, absorption was up to 17% of the administered dose after a single dose at 6 mg/kg bw and up to 7% after a single dose at 30 or 1000 mg/kg bw. Absorption may be higher by dietary administration (23% at 0.76 mg/kg bw) or by gavage in Cremophor, an emulsifying agent (33% at 6 mg/kg bw). The maximum plasma concentrations were reached after 10–48 h,

depending on the dose and the radiolabel tested. Increasing the dose by a factor of 33 resulted in an increase in the AUC of about 10-fold, correlating with the lower absorption of metaflumizone at the higher dose. Radiolabel was widely distributed throughout the body. Residues in tissues at 168 h after a single dose at 6 or 30 mg/kg bw accounted for approximately 15% or approximately 2–3% of the administered dose, respectively, with fat, liver, kidney, muscle and blood containing the highest concentrations of residues. The major route of excretion of radiolabel was via the faeces (mainly unabsorbed substance; <5% in bile), whereas less than 3% of the administered dose was excreted via the urine. The elimination half-lives depended on the position of the radiolabel, ranging from 27–48 h to 139–402 h for the B-labelled and the T-labelled metaflumizone, respectively.

Metaflumizone was metabolized via hydroxylation of the aniline or benzonitrile ring, hydrolysis of the central hydrazine carboxamide group and conjugation with sulfate, glucuronic acid, glycine or glutathione. Unchanged parent compound was the major component of the residues extracted in tissues and plasma, whereas no parent was found in the urine or bile.

Toxicological data

Metaflumizone was of low toxicity in rats exposed orally or dermally (LD_{50} >5000 mg/kg bw) and caused neither mortality nor systemic toxicity at this limit dose. Metaflumizone was also of low toxicity in rats exposed by inhalation (LC_{50} >5.2 mg/l).

Metaflumizone was not a skin irritant in rabbits, was non-irritating to slightly irritating to rabbits' eyes and was not a skin sensitizer in the guinea-pig maximization test.

After repeated administration of metaflumizone, decreased food consumption, decreased body weight gain or body weight loss and subsequent poor general state of health at higher doses were observed in all species tested. These effects were observed regardless of the route of administration (i.e. after oral, dermal or inhalation exposure). Data also indicated that female rats and dogs are relatively more sensitive than male rats and dogs to intoxication.

The poor palatability of the test substance at dietary concentrations of 50 ppm and higher was considered to significantly affect food consumption in short-term feeding studies; consequently, in all further studies (including short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, two-generation study of reproductive toxicity), the test substance was administered via gavage (rats) or in capsules (dogs). However, similar effects (e.g. decreased food consumption and body weight gain) were observed with all methods of administration, but occurred at markedly lower doses with dietary administration.

A clear mode of action for the toxicity of metaflumizone in mammals has not been identified. Many of the effects observed after repeated dosing were consistent with decreased food consumption and body weight loss but do not appear to be induced by the insecticidal mode of action (i.e. specific receptor affinity, blocking of sodium channels).

The short-term toxicity of metaflumizone was investigated in mice, rats and dogs. In two 28-day range-finding feeding studies in mice, the higher NOAEL was 50 ppm, equal to 10 mg/kg bw per day, on the basis of decreased food consumption and body weight gain at dietary concentrations of 200 ppm and above. Effects at higher doses included body weight loss, clinical signs of toxicity (ataxia, convulsions) and mortality.

In 28-day and 3-month feeding studies in rats, the NOAEL was 20 ppm, equal to 2.2 mg/kg bw per day, on the basis of decreased food consumption and body weight gain at 40 ppm, equal to 4.3 mg/kg bw per day, and above. Effects at higher doses (□1000 ppm, equal to 83 mg/kg bw per day) included body weight loss and poor general state (emaciation, hair loss, pallor, hunched posture). In subsequent 28-day and 3-month studies in rats treated by gavage, the NOAEL was 60 mg/kg bw per day on the basis of reduced food consumption and body weight gain in females at 300/200 mg/kg bw per day.

In a combined 3-month/1-year study of toxicity in dogs given metaflumizone in capsules, the NOAEL was 12 mg/kg bw per day on the basis of clinical signs of poor general state of health and premature sacrifice, decreased food consumption, reduced body weight gain and body weight loss and changes in haematological parameters at doses of 30 mg/kg bw per day and above.

Metaflumizone was tested in an adequate battery of assays for genotoxicity in vitro and in vivo. Negative results were obtained in the tests in vitro, except for a positive result in a test for chromosomal aberration in the absence of metabolic activation. In vivo, an assay for micronucleus formation in mice and a test for unscheduled DNA synthesis in rats gave negative results.

The Meeting concluded that metaflumizone was unlikely to be genotoxic.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. In an 18-month study of carcinogenicity in mice treated by gavage, the NOAEL was 250 mg/kg bw per day on the basis of decreased body weight/body weight gain and increased incidences of brown pigment in the spleen at 1000 mg/kg bw per day, the highest dose tested. There was no evidence for carcinogenicity with metaflumizone in this study.

In a 2-year study of toxicity and carcinogenicity in rats treated by gavage, the NOAEL was 30 mg/kg bw per day on the basis of increased incidences of centrilobular hepatocellular hypertrophy and hepatocellular basophilic alteration in males at 60 mg/kg bw per day. There was no evidence for carcinogenicity up to the highest doses tested (300 and 200 mg/kg bw per day for males and females, respectively).

On the basis of the absence of carcinogenicity in mice and rats and the absence of genotoxicity, the Meeting concluded that metaflumizone is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats treated by gavage, the highest dose tested (75 mg/kg bw per day) induced excessive parental toxicity, resulting in reduced fertility and high pup mortality. Consequently, the highest dose was reduced from 75 to 50 mg/kg bw per day and the intermediate dose from 30 to 20 mg/kg bw per day for the next two successive parental generations. The NOAEL for parental toxicity was 20 mg/kg bw per day on the basis of increased incidences of poor general state of health of females at doses of 30 mg/kg bw per day and above. The NOAEL for offspring toxicity was 20 mg/kg bw per day on the basis of increased incidences of stillborn pups and increased pup mortality at doses of 50 mg/kg bw per day and above. The NOAEL for effects on fertility was 50 mg/kg bw per day on the basis of a reduction in the male and female fertility indices at 75 mg/kg bw per day.

In a study of prenatal developmental toxicity in rats, the NOAEL for maternal toxicity was 40 mg/kg bw per day on the basis of reduced food consumption and decreased body weight gain at 120 mg/kg bw per day. The NOAEL for developmental toxicity was 120 mg/kg bw per day, the highest dose tested.

In a study of prenatal developmental toxicity in rabbits, the NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of clinical signs of toxicity (poor general state, including ataxia) and abortion at 300 mg/kg bw per day. The NOAEL for developmental toxicity was 100 mg/kg bw per day on the basis of decreased fetal body weights and an increased rate of incomplete ossification of the sternebrae at 300 mg/kg bw per day.

The Meeting concluded that metaflumizone caused developmental toxicity only at doses that were maternally toxic but that it was not teratogenic.

In a study of acute neurotoxicity in rats, metaflumizone did not induce signs of systemic toxicity or neurotoxicity at up to the highest dose tested (2000 mg/kg bw). In a short-term study of neurotoxicity in rats treated by gavage, the NOAELs for neurotoxicity were 300 and 150 mg/kg bw per day in males and females, respectively, the highest doses tested. The NOAEL for systemic toxicity was 36 mg/kg bw per day on the basis of poor general state, including mortality and impairment of food consumption and body weight gain at 150 mg/kg bw per day.

In a range-finding study of developmental neurotoxicity in rats treated by gavage, the NOAEL for systemic toxicity and reproductive performance was 80 mg/kg bw per day on the basis of clinical signs of poor general state and litter loss at 120 mg/kg bw per day. The concentrations of metaflumizone in milk and pup plasma were up to 15 mg/kg and up to 4 mg/kg, respectively. A full study of developmental neurotoxicity was not performed, as young rats were not more sensitive than adults to the effects of metaflumizone and no evidence of neurotoxicity was seen in standard studies of toxicity or in studies of acute or subchronic neurotoxicity.

The *Z* isomer of metaflumizone (M320I02) was of low acute oral toxicity in rats (LD_{50} >5000 mg/kg bw) and was not mutagenic in an assay for reverse mutation in bacteria. In a short-term study of toxicity in rats treated by gavage, the NOAEL was 100 mg/kg bw per day on the basis of decreased body weight gain, reduced motor activity and histopathological findings in mesenteric lymph nodes and adrenal cortex in females at doses of 300 mg/kg bw per day and above.

The main plant and soil metabolite of metaflumizone (Reg. No. 4984051; M320I23) was of low acute oral toxicity in rats (LD_{50} >2000 mg/kg bw). It was not genotoxic in assays for gene mutation in vitro, but it induced chromosomal aberrations in the presence of metabolic activation in vitro. A test for micronucleus formation in mice gave negative results. In a short-term study of toxicity in rats treated by gavage, the NOAEL was 200 mg/kg bw per day on the basis of increased salivation in both sexes and increased relative liver weights and centrilobular hepatocellular hypertrophy in females at 1000 mg/kg bw per day.

An additional soil metabolite of metaflumizone (Reg. No. 43455; M320I29) was not genotoxic in assays for gene mutation and chromosomal aberration in vitro.

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisonings with metaflumizone.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) for metaflumizone of 0–0.1 mg/kg bw based on a NOAEL of 12 mg/kg bw per day for clinical signs of poor general state of health, decreased food consumption, reduced body weight gain and body weight loss, and changes in haematological parameters at 30 mg/kg bw per day and above in a 1-year study in dogs, and using a 100-fold safety factor.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for metaflumizone in view of its low acute toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of carcinogenicity[a]	Toxicity	250 mg/kg bw per day	1000 mg/kg bw per day
		Carcinogenicity	1000 mg/kg bw per day[b]	—
Rat	Three-month study of toxicity[a]	Toxicity	60 mg/kg bw per day	200 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	30 mg/kg bw per day	60 mg/kg bw per day
		Carcinogenicity	200 mg/kg bw per day[b]	—
	Multigeneration study of reproductive toxicity[a]	Fertility	50 mg/kg bw per day	75 mg/kg bw per day
		Parental toxicity	20 mg/kg bw per day	30 mg/kg bw per day
		Offspring toxicity	20 mg/kg bw per day	50 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
	Developmental toxicity[a]	Maternal toxicity	40 mg/kg bw per day	120 mg/kg bw per day
		Embryotoxicity and fetotoxicity	120 mg/kg bw per day[b]	—
	Acute neurotoxicity[a]	Neurotoxicity	2000 mg/kg bw per day[b]	—
	Subchronic neurotoxicity[a]	Neurotoxicity	150 mg/kg bw per day[b]	—
	Developmental neurotoxicity[a]	Maternal toxicity	80 mg/kg bw per day	120 mg/kg bw per day
		Offspring toxicity	80 mg/kg bw per day	120 mg/kg bw per day
Rabbit	Developmental toxicity[a]	Maternal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Embryotoxicity and fetotoxicity	100 mg/kg bw per day	300 mg/kg bw per day
Dog	One-year study of toxicity[c]	Toxicity	12 mg/kg bw per day	30 mg/kg bw per day

LOAEL, lowest-observed-adverse-effect level
[a] Gavage administration.
[b] Highest dose tested.
[c] Capsule administration.

Estimate of acceptable daily intake for humans

0–0.1 mg/kg bw

Estimate of acute reference dose

Unnecessary

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	≤17% at 6 mg/kg bw; ≤7% at 30 or 1000 mg/kg bw
Distribution	Widely; highest concentrations in fat, liver, kidney, muscle and blood
Rate and extent of excretion	□90% within 168 h for a single dose of ≥30 mg/kg bw, mainly via faeces (<5% in bile), <3% via urine; elimination half-life, 27–48 h or 139–402 h, depending on position of radiolabel
Potential for accumulation	Evidence of accumulation in fat after repeated exposure (steady state after 21–28 days; terminal half-life, 15–17 days)
Metabolism in mammals	Extensive; hydroxylation (aniline and benzonitrile rings), hydrolysis (hydrazine carboxamide group), conjugation (sulfate, glucuronic acid, glycine, glutathione); in tissues, mainly unchanged parent
Toxicologically significant compounds (animals, plants and the environment)	Parent compound

Acute toxicity	
Rat, LD_{50}, oral	>5000 mg/kg bw
Rat, LD_{50}, dermal	>5000 mg/kg bw

Rat, LC_{50}, inhalation	>5.2 mg/l (4 h, nose-only exposure)
Rabbit, dermal irritation	Not an irritant
Rabbit, ocular irritation	Not or slightly irritating
Guinea-pig, dermal sensitization	Not sensitizing (Magnusson and Kligman test)

Short-term studies of toxicity

Target/critical effect	Decreased food consumption and body weight gain; clinical signs of poor general state, mortality or premature sacrifice; changes in haematological parameters
Lowest relevant oral NOAEL	12 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	100 mg/kg bw per day (3-month study in rats)
Lowest relevant inhalation NOAEC	0.03 mg/l (28-day study in rats)

Genotoxicity

	No genotoxic potential

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Liver (hepatocellular hypertrophy, basophilic alteration) in rats; spleen (pigment deposition), decreased body weight gain in mice
Lowest relevant NOAEL	30 mg/kg bw per day (2-year study in rats)
Carcinogenicity	No evidence for carcinogenicity in rats or mice

Reproductive toxicity

Reproductive target/critical effect	Reduced male and female fertility in the presence of severe systemic toxicity
	Increased incidences of stillborn pups and pup mortality at parentally toxic dose
Lowest relevant reproductive NOAEL	50 mg/kg bw per day for effects on fertility (two-generation study in rats)
	20 mg/kg bw per day for systemic toxicity in parents and offspring
Developmental target/critical effect	No developmental toxic effects at maternally toxic dose in rats; decreased fetal weights, incomplete ossification of sternebrae at maternally toxic dose in rabbits
Lowest relevant developmental NOAEL	100 mg/kg bw per day (rabbits)

Neurotoxicity

Acute neurotoxicity	No evidence of neurotoxicity; NOAEL: 2000 mg/kg bw (highest dose tested)
Subchronic neurotoxicity	No evidence of neurotoxicity; NOAEL: 300/150 mg/kg bw per day (highest dose tested; 90-day study in rats)

Other toxicological studies

Studies on *Z* isomer (M320I02)	Rat, oral LD_{50}, >5000 mg/kg bw
	Rat, 90-day study, NOAEL: 100 mg/kg bw per day (reduced body weight gain, histopathological changes in lymph nodes and adrenal cortex)
	No genotoxic potential

Studies on metabolites

Reg. No. 4984051 (M320I23, plant and soil metabolite)	Rat, oral LD_{50}, >5000 mg/kg bw
	Rat, 90-day study, NOAEL: 200 mg/kg bw per day (salivation, increased relative liver weight, hepatocellular hypertrophy)
	No genotoxic potential
Reg. No. 43455 (M320I29, soil metabolite)	No genotoxic potential

Medical data

	Limited data; no adverse health effects reported in manufacturing plant personnel

Summary

	Value	Study	Safety factor
ADI	0–0.1 mg/kg bw	Dog; 1-year study	100
ARfD	Unnecessary	—	—

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SPIRODICLOFEN

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Explanation

Spirodiclofen is the International Organization for Standardization (ISO)–approved name for 3-(2,4-dichlorophenyl)-2-oxo-1-oxaspiro[4.5]dec-3-en-4-yl 2,2-dimethylbutyrate (International Union

of Pure and Applied Chemistry [IUPAC]) or 3-(2,4-dichlorophenyl)-2-oxo-1-oxaspiro[4.5]dec-3-en-4-yl 2,2-dimethylbutanoate (Chemical Abstracts Service [CAS]), CAS No. 148477-71-8. Spirodiclofen is a selective, non-systemic foliar insecticide and acaricide belonging to the chemical class of ketoenols or tetronic acids, whose pesticidal mode of action is the inhibition of lipid synthesis.

Spirodiclofen is being reviewed for the first time by the present Meeting at the request of the Codex Committee on Pesticide Residues.

All pivotal toxicological studies complied with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

The toxicokinetics of spirodiclofen have been studied in rats given oral doses of radiolabelled test material. Summaries of the relevant data are presented below.

1.1 Absorption, distribution and excretion

Rats

In a GLP-compliant metabolism and disposition study, [dihydrofuranone-3-^{14}C]spirodiclofen (radiochemical purity >98%; chemical purity 99.2%) was administered to Wistar rats (four males per dose) via oral gavage as a single dose at 2 or 100 mg/kg bw or as a 14-day repeated dose of 2 mg/kg bw of non-labelled material followed by a single dose of labelled material on day 15. An additional single low dose (2 mg/kg bw) group of four female rats was also included, as well as an additional group of four males at the same dose, which was included in order to measure excretion in expired air. A group of six male rats with bile duct cannulae received a single dose of 1 mg/kg bw for assessment of biliary excretion.

Overall recovery of administered radioactivity was 89–107%. Plasma radioactivity data showed that absorption was rapid (time to peak concentration [T_{max}] values of 3–4 h for low dose and 8 h for high dose). Excretion data revealed that expired air was an insignificant route of excretion (<0.05% of the administered dose) and that both urine (57–74% of the administered dose for the low dose and 35% of the administered dose for the high dose) and faeces (23–31% of the administered dose for the low dose and 61% of the administered dose for the high dose) were major routes of elimination. Consistent with plasma kinetics data, excretion was approximately 90% complete at 48 h post-dosing. Tissue burdens were negligible (<0.2% of the administered dose in all tissues examined) and revealed no indication of sequestration or tissue specificity. Approximately 11% of the administered dose appeared in the bile, which contributed up to one third of the radioactivity excreted in the faeces. A decrease in urinary excretion of radioactivity with a commensurate increase in faecal excretion of radioactivity, combined with a comparison of area under the curve (AUC) and maximal concentration (C_{max}) values (neither of which reflected the 50-fold difference in dose), were indicative of decreased absorption at the high dose. Sex-related differences were observed, in that female rats tended to exhibit greater urinary excretion of radioactivity compared with males, whereas males excreted significantly more radioactivity via the faeces compared with females, indicating higher oral absorption in females. In addition, radioactive residues in the organs and tissues were higher in male rats than in female rats. For example, 0.138% and 0.0125% of the dose were detected in the liver and 0.010% and 0.002% in the kidney for males and females, respectively. As a whole, only 0.55% and 0.02% of the administered radioactivity remained in the body of male and female rats, respectively, after sacrifice (Table 1) (Andersch & Köster, 2000a).

Table 1. Radioactive inventory and selected pharmacokinetic parameters in rats following oral administration of [dihydrofuranone-3-^{14}C]spirodiclofen (n = 4)

Parameter	Single dose, 2 mg/kg bw	Single dose, 100 mg/kg bw	Single dose, 2 mg/kg bw	Single dose, 2 mg/kg bw	14-day repeated dose, 2 mg/kg bw per day	Single dose, 1 mg/kg bw
	Male	Male	Male	Female	Male	Male
	48 h	168 h	48 h	48 h	48 h	24 h
Expired air (% of AD)	0.05	ND	ND	ND	ND	ND
Urine (% of AD)	62.1	35.1	57.3	74.2	66.6	24.3
Faeces (% of AD)	30.9	61.3	31.1	23.3	27.6	31.3
Bile (% of AD)	ND	ND	ND	ND	ND	11.9
Carcass/organs[a] (% of AD)	0.378	0.0003	0.551	0.0156	0.150	26.2
Gastrointestinal tract (% of AD)	0.130	ND	0.0818	0.0226	0.0665	13.3
Total (% of AD)	93.6	96.3	89.0	97.5	94.5	107
C_{max} (µg/ml)	ND	51.3	2.1	2.7	2.1	ND
T_{max} (h)	ND	8.0	3.0	3.0	4.0	ND
AUC (µg·h/ml)	ND	772.5	36.7	23.7	21.2	ND

From Andersch & Köster (2000a)
AD, administered dose; ND, not determined
[a] Carcass/organs without gastrointestinal tract.

A second GLP-compliant metabolism and disposition study examined the effect of a 15-week dietary administration ("pretreatment") of unlabelled spirodiclofen to Wistar rats (four of each sex per dose) at doses of 50 or 2500 ppm, followed by a single oral gavage dose of [dihydrofuranone-3-^{14}C]spirodiclofen (radiochemical purity >98%) in carboxymethyl cellulose/acetic acid (99.5/0.5 weight per volume [w/v]) at 2 mg/kg bw. Absorption, excretion, plasma kinetics and carcass burdens were assessed over a 48 h period.

Overall recovery of the administered radioactivity was 87–103%. Analysis of plasma concentration–time course data and plasma kinetics data revealed fairly rapid absorption, as exemplified by T_{max} values of 3–4 h for both sexes in the low dose groups and males in the high dose pretreatment group. The T_{max} value in high dose treatment females was higher (8 h). Urinary excretion data and body burden data indicated that at least 58–75% of the test material was absorbed. The urine was found to be the major route of excretion, accounting for approximately 56–75% of the administered dose over a 48 h period. Elimination via the faeces accounted for approximately 22–31% of the single oral dose over the same period. Total excretion was greater than 90% over 48 h for all groups except the 2500 ppm pretreatment female group, in which total excretion was 85% over 48 h. Tissue distribution was not assessed in this study; however, mass balance data and radioactivity recovered in the skin and residual carcass indicated that little bioaccumulation occurred even after the 15-week dietary treatment. The results of this study indicated that the absorption and elimination of spirodiclofen were not significantly altered by prior dietary exposure to the test substance for 15 weeks (Table 2) (Andersch & Köster, 2000b).

In an additional GLP-compliant study designed to investigate the depletion of radioactive residues from plasma, liver, kidneys and excreta, a single oral gavage dose of [dihydrofuranone-3-^{14}C]-

Table 2. Radioactive inventory and selected pharmacokinetic parameters in rats following oral administration of [dihydrofuranone-3-^{14}C]spirodiclofen[a]

Parameter	Dietary concentration (ppm)			
	50	50	2500	2500
	Male	Female	Male	Female
Urine (% of AD)	72.0	74.7	61.4	56.4
Faeces (% of AD)	30.5	21.6	30.8	27.6
Skin (% of AD)	0.034	0.020	0.048	0.029
Carcass/organs[b] (% of AD)	0.49	0.35	1.2	1.8
Gastrointestinal tract (% of AD)	0.24	0.49	1.4	0.95
Total (% of AD)	103	97.2	94.8	86.8
C_{max} (µg/ml)	2.09	3.24	1.63	1.73
T_{max} (h)	4.0	4.0	3.0	8.0
AUC (µg·h/ml)	28.9	35.1	18.9	20.9

From Andersch & Köster (2000b)
AD, administered dose
[a] One animal from each of the male (#501) and female (#506) 2500 ppm pretreatment groups were considered outliers and were excluded from the means. For male #501: C_{max} = 0.757 µg/ml and T_{max} = 4 h; for female #506: C_{max} = 1.374 µg/ml and T_{max} = 32 h.
[b] Carcass/organs without gastrointestinal tract.

spirodiclofen (radiochemical purity >98%) in 0.5% aqueous carboxymethyl cellulose was administered to three groups of Wistar rats (four of each sex per group) at a dose of 2 mg/kg bw. The groups were sacrificed at 3, 6 and 24 h, respectively, following administration of radiolabelled spirodiclofen, and urine, blood (plasma and erythrocytes), liver, kidney, skin, gastrointestinal tract (including faeces) and carcass were collected from each animal for analysis of residues.

At 3 h post-dosing, 52% and 32% (for males and females, respectively) of the administered dose were detected in the organs and tissues of rats, whereas 44% (male) and 53% (female) were detected in the gastrointestinal tract and 1.8% (male) and 5.1% (female) in the urine. Residue levels in the organs and tissues declined over time, whereas those in the urine increased. Other than the gastrointestinal tract, which also included faeces, the highest residue concentrations were detected in the liver; levels in this organ were highest at the 3 h sampling period, after which they declined. The values in plasma were approximately 10-fold higher than those in the skin and carcass, indicating a delayed equilibrium of the radioactivity between plasma and the tissues in the rat. Residue concentrations in the kidney were comparable with those in the plasma at all time points in both sexes (Table 3) (Köster & Weber, 2002).

In two GLP-compliant rat quantitative whole-body autoradiography studies, [dihydrofuranone-3-^{14}C]spirodiclofen (radiochemical purity >98%) was administered to five male Wistar Hsd/Cpb:WU rats as a single oral (gavage) dose at 3 mg/kg bw in the first study and then to five female rats at the same dose in the second study. In each study, a single animal was sacrificed at 1, 4, 8, 24 and 48 h post-dose and subjected to quantitative whole-body autoradiography to examine the distribution of radioactivity among tissues. In addition, one control animal received unlabelled test substance and was sacrificed at 4 h.

Based on the recovery of radioactivity in the urine, the dosed radioactivity was readily absorbed and excreted, with 53–58% (in males) and approximately 51% (in females) of the dose being recovered in the urine by 24 h post-dose. Autoradiograms of longitudinal sections through the ^{14}C-treated rats showed extensive distribution of radioactivity throughout the entire body within 1 h of dosing.

Table 3. Balance of radioactivity in excreta and tissues of rats sacrificed 3, 6 and 24 h after oral administration of [dihydrofuranone-3-^{14}C]spirodiclofen

Matrix	Radioactivity (% of administered dose)					
	Males			Females		
	3 h	6 h	24 h	3 h	6 h	24 h
Urine	1.8	20.9	57.7	5.1	34.9	74.8
Erythrocytes	0.51	0.47	0.04	0.43	0.25	0
Plasma	4.0	2.6	0.20	2.8	2.1	0.02
Liver	26.0	17.9	1.5	13.5	12.4	0.09
Kidney	1.4	0.88	0.13	1.4	0.2	0.01
Carcass	12.8	10.2	0.93	9.3	7.3	0.13
Skin	7.2	5.7	0.54	4.3	3.7	0.05
Gastrointestinal tract and faeces	43.6	35.6	40.4	52.9	33.1	23.6
Total	97.3	94.3	101.3	89.8	94.5	98.7

From Köster & Weber (2002)

Data from the quantitative whole-body autoradiography indicated that concentrations of radioactivity in males were highest in the small intestines at 1 h post-dose, followed by liver and renal cortex. Although the same trend was reported for females, values were not shown for the small intestine. Based on the data provided, the highest levels at 1 h in females were noted in the liver and renal medulla. Concentrations in all other tissues (except fat and dental root in males and renal cortex and the ovary in females) were below the concentration in blood at 1 h. In females, levels peaked in the ovary at 1 h, in the urinary bladder at 4 h and in most other tissues by 8 h and had declined significantly by 24 h in all tissues. In males, levels peaked in the small intestine at 1 h and then in all other tissues at 4 or 8 h. Concentrations in most male tissues had declined by 6- to 10-fold by 24 h post-dose, with concentrations remaining highest in liver, bladder, dental root and renal cortex. Generally, levels were higher in tissues from males than in those from females. By the 48 h interval, concentrations were below the limit of detection in all tissues for females and all except liver and renal cortex in males. There was no evidence of accumulation in specific organs or tissues in this study (Table 4) (Köster, 2000, 2001).

1.2 Biotransformation

In studies of metabolism, groups of Wistar rats (four males per dose) were given [dihydrofuranone-3-^{14}C]spirodiclofen (radiochemical purity >98%) as a single oral dose at 2 or 100 mg/kg bw on its own or as a single oral dose of 2 mg/kg bw per day following a 14-day administration of unlabelled spirodiclofen at 2 mg/kg bw per day. An additional single low dose (2 mg/kg bw) group of four female rats was also included, as well as an additional group of four males at the same dose in order to measure excretion in expired air. A group of six male rats with bile duct cannulae received a single dose of 1 mg/kg bw for assessment of biliary excretion. Metabolite characterizations were conducted from animal excreta (bile, urine and faeces). Parent compound and metabolites were isolated by semipreparative high-performance liquid chromatography (HPLC) and identified by liquid chromatography–tandem mass spectrometry (LC-MS/MS) and nuclear magnetic resonance (NMR) investigations.

Analyses of excreta revealed that the test article underwent extensive metabolism. The parent compound was detected only in small quantities in the faeces, but not in the urine or bile. Identified

Table 4. Concentration of radioactivity in selected tissues or organs of rats following treatment with [^{14}C]spirodiclofen at 3 mg/kg bw (n = 1)

Tissue/organ	Concentration of radioactivity (µg equivalents/g)							
	Sacrifice interval (h post-dose)							
	1	4	8	24	1	4	8	24
	Males				Females			
Liver	1.83	5.54	5.46	0.631	0.569	1.74	2.83	0.012
Renal cortex	1.34	2.29	1.56	0.262	0.220	0.615	0.886	0.006
Renal medulla	0.240	0.734	0.882	0.066	0.506	1.84	2.93	0.021
Adrenal	0.426	1.02	0.825	0.097	0.186	0.373	0.550	<LOD[a]
Urinary bladder	0.251	3.72	6.46	0.372	1.81	7.43	6.48	0.024
Small intestine	5.69	3.82	3.00	0.092	NR	NR	NR	NR
Blood	0.471	1.52	1.28	0.140	0.189	0.427	0.657	<LOQ[b]
Brown fat	0.797	2.70	1.67	0.190	0.074	0.390	0.429	<LOD
Thyroid	0.166	0.480	0.417	0.052	0.079	0.143	0.158	<LOD
Dental root	0.485	1.40	4.01	0.304	NR	NR	NR	NR
Testis	0.019	0.171	0.139	0.015	NA	NA	NA	NA
Caput epididymis	0.044	0.162	0.204	0.014	NA	NA	NA	NA
Caudate epididymis	0.071	0.199	0.226	0.025	NA	NA	NA	NA
Preputial gland	0.177	0.673	0.878	0.030	NA	NA	NA	NA
Prostate	0.103	0.578	0.404	0.056	NA	NA	NA	NA
Ovary	NA	NA	NA	NA	0.272	0.130	0.206	<LOQ
Uterus	NA	NA	NA	NA	0.046	0.241	0.264	<LOD

From Köster (2000, 2001)

LOD, limit of detection; LOQ, limit of quantification; NA, not applicable; NR, not reported

[a] LOD established as 2 times the background radioactivity multiplied by the calibration factor derived from the microstandards used.

[b] LOQ set as either a) 2 times the respective LOD value or b) the lowest value of the calibration microstandards that were included in the calibration curve, whichever was higher.

components in urine and faeces accounted for approximately 59–90% of the administered dose, depending on dose regimen. Ten urinary and 11 faecal components were characterized. With few exceptions, metabolite profiles were generally similar qualitatively but varied quantitatively between dose groups. Greater levels of parent compound were detected in faeces from the high dose group, which was consistent with the observed decreased absorption. The sex-related difference in absorption/excretion processes in the single low dose group was reflected quantitatively and to some extent qualitatively in the metabolite profiles. The predominant metabolite in the excreta of females was the enol metabolite (58% of the administered dose), followed by the 4-hydroxy-enol (16%) and the 3-hydroxy-enol (5%) (equatorial isomers [eq.]). In low-dose males, the major metabolites in excreta were the 3-hydroxy (eq.)-enol (28–33%), the 4-hydroxy (eq.)-enol (18–22%) and the enol (8–9%). The quantitative metabolic profile shifted slightly for high-dose males, with the enol, the 3-hydroxy (eq.)-enol and the 4-hydroxy (eq.)-enol representing approximately 22%, 14% and 9% of the administered dose, respectively. An assessment of the time course of excretion of metabolites via the faeces revealed that most of the metabolites (generally >90%) were excreted during the 0–24 h period following dosing. Qualitatively, the biliary metabolite profile closely paralleled that of the urine, with the exception of a glucuronide conjugate in the bile, which represented approximately one third of the radioactivity in the bile over a 24 h period following dosing. The metabolite profile

in the bile also lacked several components (glyoxylic acid, dichloromandelic acid, dichlorobenzoic acid, mandelic acid cyclohexyl ester) that were detected in the faeces; these were likely the result of biotransformation processes within the gut.

An additional study examined the effect of 15-week dietary pretreatment with spirodiclofen at 50 or 2500 ppm, followed by a single oral gavage dose of [dihydrofuranone-3-^{14}C]spirodiclofen (radiochemical purity >98%) at 2 mg/kg bw to groups of Wistar rats (four of each sex per dose). Metabolites in urine and faeces (at 48 h) were identified based on HPLC investigations. The dietary pretreatment did not appear to significantly affect the metabolism of spirodiclofen in the rat. Similar to the other study, no parent compound was detected in the urine, whereas nine metabolites were characterized. In the faeces, parent compound accounted for 0.38–13.5% of the administered dose, whereas 12 metabolites accounted for 0.17–8.95% (each) of the dose. Sex-related differences were observed that were independent of the pretreatment regimen. The most notable difference was that the enol metabolite in urine accounted for approximately 5% of the administered radioactivity in male rats, compared with 40–52% in female rats. In male rats, the 3-hydroxy-enol was the major metabolite (~23–34% versus 3.5–4.5% in females). Other minor quantitative and qualitative sex-related differences were observed in metabolites that each represented less than 5% of the administered radioactivity.

Major metabolites (i.e. the enol, the 3-hydroxy-enol [equatorial and axial isomers] and the 4-hydroxy-enol [equatorial and axial isomers]) were characterized in urine and plasma, as well as in liver and kidney extracts, in an additional study using three groups of Wistar rats (four of each sex per group) administered a single oral gavage dose of [dihydrofuranone-3-^{14}C]spirodiclofen (radiochemical purity >98%) in 0.5% aqueous carboxymethyl cellulose. The groups were sacrificed at 3, 6 and 24 h, respectively, and the major metabolites were quantified in the various matrices. These investigations were conducted to further elucidate the metabolism of spirodiclofen, as there were sex differences noted in the amounts of these major metabolites in previous studies conducted. These investigations confirmed the previously noted sex differences. In urine, the enol metabolite was dominant in females, whereas the hydroxylated enol metabolites were the major metabolites in males. Metabolite levels increased over time from 3 to 24 h in urine samples. In plasma, liver and kidney samples, the enol was the major metabolite quantified in both sexes. However, the hydroxylated enols were present in higher amounts in males. In females, the hydroxylated enols were not detected at all in plasma and only at very low levels in liver (<1.3% of total radioactive residues) and kidney (<5% of total radioactive residues). In plasma, liver and kidney samples, metabolite levels declined over the 24 h sampling period.

Based on the metabolism studies conducted, it was concluded that the metabolism of [dihydrofuranone-3-^{14}C]spirodiclofen in the rat generally proceeds via formation of an enol that undergoes hydroxylation or is metabolized to a mandelic acid derivative and dichlorobenzoic acid (Figure 1) (Andersch & Köster, 2000a,b; Köster & Weber, 2002).

2. Toxicological studies

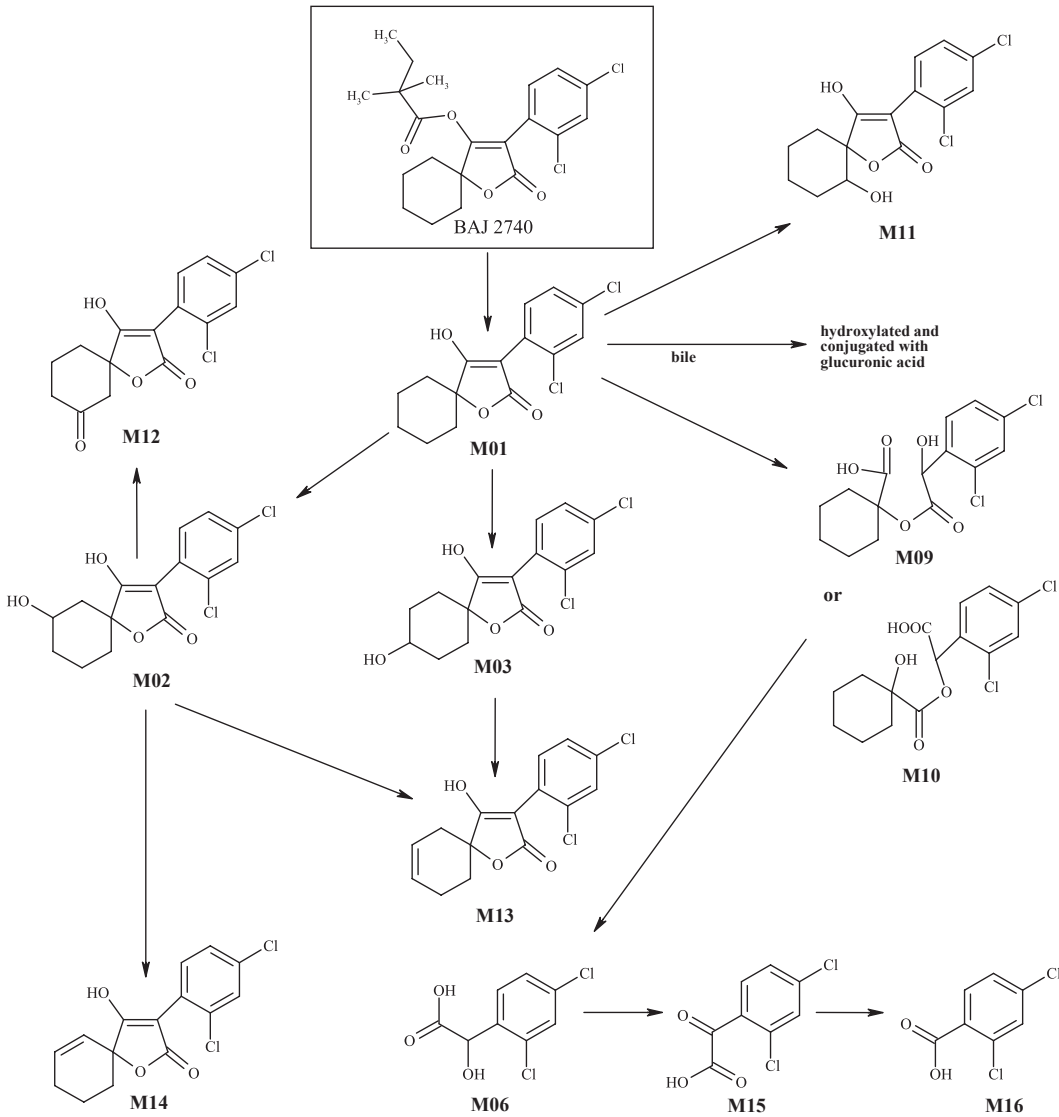
2.1 Acute toxicity

(a) Lethal doses

The results of the acute toxicity studies with spirodiclofen are summarized in Table 5. All studies complied with GLP.

Spirodiclofen is of low acute toxicity via the oral, dermal and inhalation routes. No clinical signs of toxicity or other treatment-related findings were noted in any of the acute toxicity studies.

Figure 1. Proposed metabolic pathway of spirodiclofen (BAJ 2740) in rats



M01: BAJ 2740-enol
M02: 3-hydroxy (eq.)-BAJ 2740-enol / 3-hydroxy (ax.)-BAJ 2740-enol
M03: 4-hydroxy (eq.)-BAJ 2740-enol / 4-hydroxy (ax.)-BAJ 2740-enol
M06: 2,4-dichloromandelic acid
M09: 2,4-dichloromandelic acid cyclohexyl ester (isomer 1)
M10: 2,4-dichloromandelic acid cyclohexyl ester (isomer 2)
eq. equatorial
ax. axial

M11: 2-hydroxy (eq.)-BAJ 2740-enol
M12: 3-keto-BAJ 2740-enol
M13: 3-ene-BAJ 2740-enol
M14: 2-ene-BAJ 2740-enol
M15: 2,4-dichloroglyoxylic acid
M16: 2,4-dichlorobenzoic acid

From Kaune, Temerowski & Krautstrunk (2008)

Table 5. Acute toxicity of spirodiclofen[a]

Species	Strain	Sex	Route	Vehicle	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Reference
Rat	HsdCpb:WU Wistar	M & F	Oral	Carboxymethyl cellulose sodium salt	>2500[b]	—	Kroetlinger (1996a)
		M & F	Dermal	Water[c]	>2000	—	Kroetlinger (1996b)
		M & F	Inhalation	None[d]	—	>5.03	Pauluhn (1997)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male

[a] Spirodiclofen of >99% purity was used in the acute studies.

[b] The reported LD_{50} was based on the Organisation for Economic Co-operation and Development (OECD) draft guideline "Acute Oral Toxicity—Acute Toxic Class Method" from 1995. Based on the revised OECD test guideline 423 (adopted in 2001), the company indicated that the interpretation would be as follows: LD_{50} oral >2000 mg/kg bw, equivalent to category "unclassified" of the Globally Harmonized System of Classification and Labelling of Chemicals, and LD_{50} cut-off >5000 mg/kg bw.

[c] The test substance was mixed with water to form a paste.

[d] The test substance was administered as an aerosolized powder.

(b) Dermal irritation

In a dermal irritation study performed according to Organisation for Economic Co-operation and Development (OECD) test guideline 404, three male Himalayan rabbits were administered 500 mg of spirodiclofen (purity 99.1%; moistened with water) dermally to a 6 cm^2 area of intact skin on the trunk of each animal for 4 h. Animals were observed for 3 days, and dermal reactions were evaluated according to the Draize method at 1, 24, 48 and 72 h following patch removal. Statements of adherence to quality assurance (QA) and GLP were included. No signs of dermal irritation were observed (Leuschner, 1997a).

(c) Ocular irritation

In an eye irritation study performed according to OECD test guideline 405, 100 mg of spirodiclofen (purity 99.1%) was instilled into the conjunctival sac of the right eye of three young adult male Himalayan rabbits for 24 h. The untreated left eye served as the control. Animals were observed for 3 days, and irritation was scored by the method of Draize at 1, 24, 48 and 72 h following administration. Statements of adherence to QA and GLP were included. No signs of irritation were noted at any point during the study (Leuschner, 1997b).

(d) Dermal sensitization

In a GLP-compliant dermal sensitization study conducted according to the Magnusson and Kligman guinea-pig maximization test (OECD test guideline 406), spirodiclofen (purity 99.1%) in physiological saline containing 2% Cremophor EL was tested using young adult female Hsd Poc:DH guinea-pigs. Animals were divided into three groups consisting of an irritation screening group (5 animals), a test group (10 animals) and a control group (5 animals). Based on the results of the irritation screening studies, the test material was administered at 5% for the intradermal injection (0.1 ml) and 50% for the topical induction (0.5 ml) as well as for the challenge applications (0.5 ml). A positive control study using 2-mercaptobenzothiazole (used at 2.5% for intradermal inductions and 40% for the topical induction and challenge treatments), a known skin sensitizer, formulated with physiological sodium chloride containing 2% by volume (v/v) Cremophor EL, was conducted within 6 months of the conduct of this study. Results of the study demonstrated the ability of the performing laboratory to identify a dermal sensitizer using the guinea-pig maximization method.

The first induction phase involved three 0.1 ml paired intradermal injections consisting of complete Freund's adjuvant diluted 1:1 with sterile physiological saline solution, 5% spirodiclofen

in vehicle and 5% spirodiclofen in equal parts vehicle and complete Freund's adjuvant. The animals of the control group were treated in the same manner as the test group, replacing the test substance with vehicle. One week later, a topical induction was performed with 0.5 ml of 50% spirodiclofen in the vehicle for a 48 h exposure period. The challenge was performed 3 weeks after the intradermal induction. A 0.5 ml topical application of 50% spirodiclofen in vehicle was administered for 24 h to test animals; control animals were treated with vehicle only. A patch loaded only with the vehicle was placed on the left flank of control animals. Skin reactions were assessed 48 and 72 h after the start of the application in accordance with the Buehler scoring scale. A second challenge was performed in the same way, with the exception that the applications were made to the opposite side of the animals.

Following the topical induction, open wounds were noted at the treatment site on day 9. These sores were healed by day 17, and no other irritation was noted. Skin reactions were observed in 4 of 10 animals (40%) in the test group and 0 of 10 animals in the control group following both the first and second challenges. Only one animal showed a positive reaction after both challenge treatments. It is concluded that spirodiclofen is a potential dermal sensitizer under the conditions of the guinea-pig maximization test. However, it should be noted that the interpretation of the test result is limited by the fact that only one animal showed a positive reaction following both challenge treatments (Stropp, 1996).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a GLP-compliant short-term feeding study, spirodiclofen (purity 99.1%) was administered to CD-1 mice (10 of each sex per dose) in the diet (which had been premixed with 1% peanut oil) at dose levels of 0, 100, 1000 or 10 000 ppm (equal to 0, 15, 164 or 1640 mg/kg bw per day for males and 0, 30, 234 or 2685 mg/kg bw per day for females) for 13 weeks. Clinical signs were assessed daily, and body weights, food consumption and water intake were recorded weekly. Blood was collected for haematology and clinical chemistry investigations on weeks 12/13 and 13/14, respectively. Ophthalmological examinations and urinalysis were not conducted. At study termination, all animals were subjected to gross necropsy, and the liver, adrenal glands and all necropsy findings were examined microscopically. In addition, the kidneys, testes, epididymides, spleen, thyroid gland and intestines from control and high-dose animals were also examined microscopically. Livers, spleen, kidney, testes, ovary, brain and adrenal glands from all animals were weighed.

There were no treatment-related effects noted for mortality, clinical signs, body weights, body weight gains, food or water consumption or haematological parameters. Cholesterol levels were decreased at 10 000 ppm (by 31% for females and by 18% for males); these decreases attained statistical significance for females only. Histopathological examinations revealed effects on the liver and adrenal cortex. Centrilobular hepatocellular hypertrophy was observed in males at 100 ppm and above and in females at 10 000 ppm, and periportal cytoplasmic vacuolation was noted in males and females at 10 000 ppm. Cytoplasmic vacuolation of the adrenal cortex occurred in males at 10 000 ppm and in females at 1000 ppm and above. In 9 out of 10 females at 10 000 ppm, this alteration was increased in severity, frequently leading to a degeneration of cortical cells accompanied by mononuclear infiltrates. Kidney weights were also decreased for males at 10 000 ppm. Originally, no findings were noted in male reproductive tissues. However, due to histopathological lesions that were observed in dogs treated with spirodiclofen, male reproductive tissues (testes, epididymides, prostates, seminal vesicles) from mice in this study were re-examined as an addendum to the main study. This re-examination revealed an increased incidence of hypertrophic Leydig cells in the testes

Table 6. Incidences of selected microscopic findings in the mouse

Histopathological finding	Incidence (no. of animals with finding)			
	Dietary concentration (ppm)			
	0	100	1000	10 000
Males (*n* = 10)				
Liver – centrilobular hepatocellular hypertrophy	0	3	3	6
Liver – periportal cytoplasmic vacuolation	0	0	0	1
Adrenal – cytoplasmic vacuolation[a]	0	0	0	8
Adrenal – degeneration of cortical cells	0	0	0	0
Adrenal – mononuclear infiltrate	0	0	0	0
Testes – Leydig cell hypertrophy	1	1	9	10[b]
Testes – Leydig cell vacuolation	0	0	0	7
Testes – degeneration of germinal epithelium	0	2	0	0
Females (*n* = 10)				
Liver – centrilobular hepatocellular hypertrophy	0	0	0	2
Liver – periportal cytoplasmic vacuolation	0	0	0	3
Adrenal – cytoplasmic vacuolation	0	0	6	10
Adrenal – degeneration of cortical cells	0	0	0	9
Adrenal – mononuclear infiltrate	0	0	0	9

From Leser & Romeike (1997, 1998); Leser & Hartmann (2002)
[a] Only nine examined.
[b] Increased severity compared with other dose levels and controls.

at 1000 ppm and above (incidence of 1/1/9/10), which was characterized by a foamy cytoplasm of the enlarged Leydig cells and was located in the intertubular interstitium and beneath the capsule. At 10 000 ppm, hypertrophy was increased in severity and was accompanied by a minimal to slight vacuolation of the Leydig cells in 7 out of 10 animals. No findings were noted in the prostate or seminal vesicles (Table 6).

The no-observed-adverse-effect level (NOAEL) was 100 ppm (equal to 30 mg/kg bw per day) for females, based on cytoplasmic vacuolation of the adrenal cortex at 1000 ppm. For males, the study authors did not establish a NOAEL owing to the hepatocellular hypertrophy noted at 100 ppm (equal to 15 mg/kg bw per day), and therefore this dose was considered to be the lowest-observed-adverse-effect level (LOAEL) for males. However, the Meeting considered that the findings of centrilobular hepatocellular hypertrophy in male mice at 100 ppm were adaptive and therefore non-adverse. As such, the NOAEL for males was also identified to be 100 ppm (equal to 15 mg/kg bw per day) based on an increased incidence of Leydig cell hypertrophy at 1000 ppm (Leser & Romeike, 1997, 1998; Leser & Hartmann, 2002).

Rats

In a GLP-compliant 4-week feeding study, spirodiclofen (purity 98.2%) was administered to female Wistar rats (five per dose) via diet (containing 1% peanut oil) at dose levels of 0, 100, 500 or 5000 ppm (equal to 0, 10, 49.6 and 569.3 mg/kg bw per day, respectively) for 4 weeks. Clinical observations were performed daily, and body weights and food and water consumption were determined weekly. Clinical laboratory examinations and gross necropsy (with organ weights and tissue sampling) were performed on all surviving animals at termination. Induction of hepatic enzymes was determined, and cell proliferation of hepatic and renal tissues was assessed immunohistochemically

Table 7. Selected clinical chemistry findings and liver enzyme levels in the female rat

Parameter	Dietary concentration (ppm)			
	0	100	500	5000
Plasma levels				
ASAT (U/l)	34.9	36.6	32.0	43.1**
ALAT (U/l)	41.6	45.4	37.7	54.7*
AP (U/l)	403	371	364	1260
Total protein (g/l)	64.5	63.9	62.9	59.2**
Triglyceride (mmol/l)	0.76	1.11*	0.76	0.40*
Cholesterol (mmol/l)	2.07	2.06	1.93	1.08**
Liver tissue levels				
ECOD (nmol/g per minute)	1.5	1.6	1.9*	2.3*

From Kroetlinger & Geiss (2000)
ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; U, units; * $P < 0.05$; ** $P < 0.01$

(using the proliferating cell nuclear antigen technique). Organs and tissues were microscopically examined. Immunotoxicological investigations were also performed, which included measures of cell counts in the spleen, lymph nodes and bone marrow, as well as assessments of subpopulations of spleen and lymph node cells, macrophage activity and responsiveness of the spleen and lymph node cells (by FACScan analyses). Serum antibody titres (immunoglobulins [Ig] IgG, IgM, IgA) were also determined.

There were no treatment-related effects on mortality, clinical signs, body weight, food and water consumption or any of the haematological parameters tested. Treatment-related effects were observed in clinical chemistry of animals receiving 5000 ppm and included increases in aminotransferase and alkaline phosphatase (AP) activities and decreases in total protein, triglyceride and cholesterol levels. While these parameters may be considered incidental on their own, taken together, the study authors suggested that they indicate an effect of treatment on the liver. Biochemical determinations of hepatic tissue showed induction of a phase I, cytochrome P450–dependent mono-oxygenase enzyme (7-ethoxycoumarin deethylase [ECOD]; increased by 53%) at 5000 ppm (Table 7). The study authors indicated that the determination of cell counts in the spleen, mesenteric lymph node and femoral bone marrow and mitogen stimulation did not result in treatment-related effects. They also concluded that FACScan analyses revealed a decrease in splenic T cells after treatment with 5000 ppm and that titres of IgG antibodies were reduced after treatment with 500 and 5000 ppm. The effects on the immune system were considered by the study authors to occur at doses that caused signs of significant toxicity and were so slight that they were not considered to represent adverse immunotoxic effects.

The Meeting concluded that the results of the immune system tests were inconclusive, based on the following considerations: 1) the FACScan analyses were done in pooled (group) samples (in addition, the study report stated that these data were compared with historical control data in order to establish whether the findings were "significant"; however, these historical control data were not provided); 2) only graphs were submitted of antibody (IgG, IgA, IgM) titres; 3) the immunotoxicity report stated that there was an unusually high variation in controls for the IgA titre, size distributions of lymph node cells in the FACScan analyses at 100 ppm as well as all spleen cell counts, which prevented the detection of dose-related effects for these parameters; and 4) no individual data were submitted to validate the results.

Gross pathological and histopathological examinations did not reveal any treatment-related changes. Cell proliferation studies revealed no effect on hepatic cells; however, increases noted in

kidney cell proliferation in both the medulla and the cortex were considered to reflect a toxic effect of treatment.

The NOAEL in this study was 500 ppm (equal to 49.6 mg/kg bw per day), based on clinical chemistry findings (increases in aminotransferase and AP activities and decreases in total protein, triglyceride and cholesterol levels) and liver enzyme induction at 5000 ppm. The Meeting noted that the study was stated to have been conducted in accordance with OECD test guideline 407 (adopted in 1995); however, the study is not acceptable according to this guideline, owing to the use of only one sex (five females per group). According to the guideline, five animals of each sex per group should be used (Kroetlinger & Geiss, 2000).

In a GLP-compliant 14-week feeding study, spirodiclofen (purity 99.1%) was administered to Wistar rats (10 of each sex per dose) via the diet (containing 1% peanut oil) at dose levels of 0, 100, 500, 2500 or 12 500 ppm (equal to 0, 6.6, 32.1, 166.9 and 851.4 mg/kg bw per day for males and 0, 8.1, 47.1, 215.3 and 995.8 mg/kg bw per day for females, respectively) for 14 weeks. Additional groups of 10 males and 10 females were treated with 0 or 12 500 ppm of spirodiclofen (equal to 0 or 902.2 mg/kg bw per day for males and 0 or 1094.7 mg/kg bw per day for females) for 14 weeks and then observed for reversible effects over 4 weeks. A satellite group of rats (five of each sex per dose) was dosed for 4 weeks with the same doses of spirodiclofen as in the main study for immunotoxicological investigations. Clinical observations were performed daily, and body weights and food consumption were determined twice weekly. Ophthalmological examinations were performed on all animals of all groups before the start of the study and at week 11. Clinical chemistry, haematology and urinalysis examinations were conducted on blood or urine samples from weeks 5 and 13 in the main study and from week 17 in the recovery group. Levels of thyroid hormones (triiodothyronine [T_3], thyroxine [T_4] and thyroid stimulating hormone [TSH]) in plasma were also measured at these time points. Gross necropsy (with organ weights and tissue sampling) and histopathological examinations were performed on all animals in both the main study and recovery groups. Four days prior to necropsy, animals in the satellite group were immunized in vivo with sheep red blood cells, and a plaque-forming cell assay (PFCA) was performed. Other immunotoxicological investigations included measures of cell counts in the spleen, lymph nodes and bone marrow (methods not reported) and assessments of subpopulations of spleen and lymph node cells (by FACScan), macrophage activity in spleen and mesenteric lymph nodes following phorbol myristate acetate stimulation as well as serum antibody titres (IgG, IgM, IgA) using enzyme-linked immunosorbent assay.

There were no treatment-related effects on mortality, clinical signs, ophthalmoscopic examinations or water intake. During the treatment period, statistically significant decreases in body weights were observed at 12 500 ppm in both sexes (by 13% in males and by 12% in females). Slight decreases in body weights were also observed in both sexes at 2500 ppm (by 4% in males and by 7% in females). During the recovery period, the decreases in body weights in the 12 500 ppm group became less apparent (decreased by 7% in males and by 5% in females). Food consumption was comparable to that of controls during treatment up to and including 500 ppm in both sexes. At 2500 and 12 500 ppm, it was significantly reduced in the first determination period (possibly due to adaptation) and occasionally during further treatment at 12 500 ppm in both sexes. During the recovery period, food intake in females was increased.

Increased erythrocyte counts and reduced mean corpuscular volume and mean corpuscular haemoglobin values were noted in females at 12 500 ppm during treatment and after the recovery period. These differences were slight and were not regarded as indicative of primary haematotoxicity. Significant decreases in leukocyte counts in the 12 500 ppm group of both sexes, decreases in thrombocyte counts in males at 12 500 ppm and slight but statistically significant increases in thromboplastin times at 2500 and 12 500 ppm for both sexes were also noted. The increases in thromboplastin times were not considered biologically relevant. After the recovery period, the blood coagulation

parameters were comparable to those of controls. Clinical chemistry showed significant increases in AP activity at 2500 and 12 500 ppm (both sexes) and in asparate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activities at 12 500 ppm (both sexes). Decreases in glutamate dehydrogenase (GLDH) activity were also noted in both sexes, but were not always dose dependent. Significantly decreased plasma cholesterol and triglyceride levels were observed at 12 500 ppm in both sexes and at 2500 ppm in males only. Significantly decreased serum phosphate concentrations were noted at 12 500 ppm in both sexes. Differences in ALAT activity, AP activity, cholesterol levels and triglyceride levels were still noted at the end of the recovery period in males. Plasma levels of TSH were slightly increased in females at 2500 and 12 500 ppm. After the recovery period, higher T_3 and T_4 and lower mean TSH concentrations were determined for males. As differences were slight and did not result in histological changes, they were not considered to be toxicologically relevant (Table 8). Increases in absolute and relative adrenal weights were observed at 12 500 ppm (both sexes), which correlated with microscopic findings in the adrenal gland. These consisted of an increase in severity of uniformly small cytoplasmic vacuolation in the cortex of males at doses of 2500 ppm and above and in females at 500 ppm and above. At the high dose, an increase in severity of mixed cytoplasmic vacuolation in the adrenal cortex was also noted. Other histopathological findings included minimal to moderate vacuolation in the small intestine (mostly the jejunum) at 2500 ppm and above (both sexes) and reduced glycogen content of hepatocytes in four high-dose females (Table 9). After the recovery period, there were no toxicologically relevant differences in organ weights or histopathology.

The study authors indicated that the immunotoxicity investigations revealed slight decreases in cell counts (males at 12 500 ppm) and subpopulation changes (males at 2500 ppm and both sexes at 12 500 ppm) in the spleen. In addition, the study authors noted decreases in IgM and IgG antibodies for females at 12 500 ppm in the PFCA, and serum IgA antibody titres were also slightly reduced in females at 2500 ppm and above. Absolute and relative spleen (females) and thymus weights (males) were reduced at 12 500 ppm. The Meeting considered that the results of the immunotoxicity tests were inconclusive based on the following considerations: 1) no individual animal data were submitted, and thus the results could not be validated; 2) the FACScan analysis for lymphocyte subpopulations was performed on pooled samples, and the findings were difficult to interpret; 3) results of the macrophage activity, PFCA and antibody titres were presented in graphs only, and no individual data were submitted; and 4) the report stated that "regarding the PFCA for male rats it is obvious that the animals were not stimulated properly", and therefore the accuracy of the results of the PFCA is questionable. As the immunotoxicity testing is not part of the guideline requirement, elimination of these data does not affect its acceptability for a guideline subchronic study.

The NOAEL for females was determined to be 100 ppm (equal to 8.1 mg/kg bw per day), based on cytoplasmic vacuolation of the adrenal cortex at 500 ppm, whereas the NOAEL for males was 500 ppm (equal to 32.1 mg/kg bw per day), based on clinical chemistry findings (increased AP and decreased cholesterol and triglyceride levels) and vacuolation of the adrenal cortex and the small intestine at 2500 ppm (Wirnitzer & Romeike, 1998; Wirnitzer & Hartmann, 2002a).

Dogs

In a 4-week feeding dose range–finding study, spirodiclofen (purity 99.1%) was administered to groups of Beagle dogs (two of each sex per dose) in the diet (mixed with water to form a paste) at dose levels of 0, 400, 2000 or 10 000 ppm (equal to 0, 11.3, 63.5 and 284.5 mg/kg bw per day for males and females combined) for 4 weeks. Although this study did not comply with GLP, it was performed in a GLP-certified laboratory and followed general GLP procedures. Clinical observations were conducted twice daily, food and water intake were assessed daily and body weights were recorded weekly. Reflexes were assessed, and body temperature and pulse were recorded before the start of the study and at week 4. Blood was collected for haematological and clinical chemistry investigations at pretest and during weeks 2 and 4. Levels of plasma T_3 and T_4 and thyroxine-binding capacity were

Table 8. Selected clinical chemistry findings in the rat

Clinical chemistry parameter	Dietary concentration (ppm)					Historical control range[a]
	0	100	500	2500	12 500	
Males						
Week 5						
Cholesterol (mmol/l)	2.26	2.18	2.02	1.60**	1.15**	
Triglyceride (mmol/l)	1.67	1.75	1.36	0.90**	0.58**	
T_3 (nmol/l)	1.41	1.62	1.61	1.56	1.53	1.35–1.78
T_4 (nmol/l)	59	59	65	64	69*	63–67
TSH (µg/l)	3.75	4.04	6.05	4.04	3.31	
Week 13						
Cholesterol (mmol/l)	2.07	2.04	2.11	1.63**	1.29**	
Triglyceride (mmol/l)	1.79	1.45	2.16	1.22	0.80**	
T_3 (nmol/l)	1.28	1.41	1.44	1.34	1.35	1.28–1.74
T_4 (nmol/l)	56	52	56	54	59	40–65
TSH (µg/l)	2.79	3.42	4.14	3.97	3.71	
Recovery						
Cholesterol (mmol/l)	2.25	—	—	—	1.92*	
Triglyceride (mmol/l)	2.13	—	—	—	1.45**	
T_3 (nmol/l)	1.39	—	—	—	1.63**	1.23–1.85
T_4 (nmol/l)	58	—	—	—	65*	43–59
TSH (µg/l)	4.42	—	—	—	1.95*	
Females						
Week 5						
Cholesterol (mmol/l)	2.01	2.01	1.80	1.71	1.17**	
Triglyceride (mmol/l)	1.40	1.30	0.92*	0.61**	0.46**	
T_3 (nmol/l)	1.65	1.63	1.69	1.52	1.56	1.31–1.72
T_4 (nmol/l)	48	47	50	52	56	48–61
TSH (µg/l)	1.39	1.21	1.29	1.99	2.96**	
Week 13						
Cholesterol (mmol/l)	2.04	2.17	1.88	1.79	1.24**	
Triglyceride (mmol/l)	1.27	1.35	1.19	0.85	0.41**	
T_3 (nmol/l)	1.39	1.48	1.49	1.47	1.46	1.31–1.69
T_4 (nmol/l)	42	44	46	46	45	39–49
TSH (µg/l)	0.84	1.36*	1.25	2.33**	2.87**	
Recovery						
Cholesterol (mmol/l)	1.87	—	—	—	2.02	
Triglyceride (mmol/l)	1.54	—	—	—	1.15	
T_3 (nmol/l)	1.45	—	—	—	1.45	1.42–1.68
T_4 (nmol/l)	44	—	—	—	42	37–50
TSH (µg/l)	1.23	—	—	—	1.44	

From Wirnitzer & Romeike (1998); Wirnitzer & Hartmann (2002a)

* $P < 0.05$; ** $P < 0.01$

[a] Historical control data for T_3 and T_4 collected from five subchronic rat studies ($n = 10$ for each sex) conducted between 1991 and 1994.

Table 9. Incidences and severity of selected microscopic findings in the rat

Histopathological finding	Incidence (no. of animals with finding) / severity[a]				
	Dietary concentration (ppm)				
	0	100	500	2500	12 500
Males					
Main study (n = 10)					
Jejunum – epithelial vacuolation	0	0	0	8 / 1.6	8 / 1.9
Adrenal – small cortical vacuolation	5 / 1.4	9 / 1.7	8 / 1.4	10 / 3.3	10 / 3.3
Adrenal – mixed cortical vacuolation	7 / 2.3	7 / 1.7	6 / 1.5	9 / 1.3	10 / 2.9
Recovery (n = 10)					
Adrenal – small cortical vacuolation	8 / 2.0	—	—	—	8 / 2.3
Adrenal – mixed cortical vacuolation	6 / 2.3	—	—	—	5 / 1.6
Females					
Main study (n = 10)					
Jejunum – epithelial vacuolation	0	0	0	7 / 2.3	7 / 2.4
Adrenal – small cortical vacuolation	4 / 1.5	3 / 1.7	8 / 1.8	8 / 2.0	10 / 3.8
Adrenal – mixed cortical vacuolation	0	0	0	0	0
Liver – reduced glycogen content	0	0	0	0	4
Recovery (n =10)					
Adrenal – small cortical vacuolation	7 / 1.9	—	—	—	7 / 2.0
Adrenal – mixed cortical vacuolation	0	—	—	—	0

From Wirnitzer & Romeike (1998); Wirnitzer & Hartmann (2002a)

[a] The severity scores represent the average score for each group, based on the following scale: Grade 1: Minimal/very few/very small; Grade 2: Slight/few/small; Grade 3: Moderate/moderate number/moderate size; Grade 4: Marked/many/large; Grade 5: Massive/extensive number/extensive size.

included in these investigations. Urinalysis was conducted at the same time points. Gross necropsy (including organ weight assessments) and microscopic examinations were carried out at study termination. In addition, liver triglyceride levels and activities of the following enzymes were determined: microsomal liver enzymes (aminopyrine-*N*-demethylase [N-DEM] and *p*-nitroanisole-*O*-deethylase [O-DEM]), cytochrome P450, cytochrome P450–dependent mono-oxygenases (ECOD, 7-ethoxyresorufin *O*-deethylase [EROD], aldrin epoxidase [ALD]), epoxide hydrolase (EH) and the conjugation enzymes (glutathione *S*-transferase [GST], uridine diphosphate (UDP)–glucuronyltransferase [GLUT]).

There were no compound-related effects on mortality, clinical signs, body weight, food and water consumption, urinalysis or haematological parameters assessed. Increased plasma activities of ASAT, ALAT, AP and lactate dehydrogenase were observed in 10 000 ppm male and female dogs in a time-dependent manner. One female at 2000 ppm also showed an increased ASAT activity at weeks 2 and 4. GLDH levels appeared to be increased for high-dose males, and T_4 levels were slightly decreased for both sexes, beginning at 2000 ppm, in a time-dependent manner. Cholesterol levels were reduced at 10 000 ppm in both sexes at weeks 2 and 4. Serum protein, albumin and iron levels were also decreased at the high dose. A dose-dependent increase in microsomal liver enzymes (O-DEM and N-DEM) was observed in animals at doses of 2000 ppm and above. The cytochrome P450–dependent mono-oxygenases (ECOD and ALD) were also induced by spirodiclofen at doses of 2000 ppm and above, and there was a marginal increase noted in GLUT activity at the high dose. Increased relative adrenal weights were observed at 2000 ppm and above, and increased relative kidney weights

Table 10. Incidences of selected microscopic findings in the dog

Histopathological finding	Incidence (no. of animals with finding)			
	Dietary concentration (ppm)			
	0	400	2000	10 000
Males (*n* = 2)				
Adrenal – cytoplasmic vacuolation	0	0	2	2
Jejunum – vacuolation of epithelium	0	0	1	2
Liver – periportal single-cell necrosis	0	0	0	2
Testes – Leydig cell vacuolation	0	0	2	2
Testes – Leydig cell hypertrophy	0	0	0	1
Testes – immature	0	0	0	1
Prostate – immature	0	0	0	1
Epididymides – oligospermia/aspermia	0	0	0	1
Females (*n* = 2)				
Adrenal – cytoplasmic vacuolation	1	1	2	2
Jejunum – vacuolation of epithelium	0	0	0	1
Liver – periportal single-cell necrosis	0	0	0	2

From Wetzig, Romeike & Sander (2001)

were observed at 10 000 ppm. Although there was a slight, dose-dependent increase in relative liver weight, values from all groups were within historical control ranges. Histopathological examinations revealed that all high-dose animals showed minimal to slight periportal single-cell necrosis in the liver. In the adrenal glands, animals dosed at 2000 ppm and above showed an increased cytoplasmic vacuolation in the cortex. In the intestine (jejunum), a moderate to marked vacuolation of superficial mucosal epithelial cells was found in animals dosed at 2000 ppm and higher. In the testis, vacuolation of Leydig cells was found in males dosed at 2000 ppm and above. In one high-dose animal, this finding was combined with hypertrophy/activation of the Leydig cells. Furthermore, testes and prostate of this animal were considered immature. The epididymides of one male also showed a massive oligospermia/aspermia and slight spermatic debris (Table 10). No correlates to the increased kidney weights were seen in the clinical chemistry, urinary or histopathological assessments.

The NOAEL is 400 ppm (equal to 11.3 mg/kg bw per day, both sexes combined), based on liver enzyme induction, increased adrenal weights and histopathological findings in the adrenal gland and testes at 2000 ppm (Wetzig, Romeike & Sander, 2001).

In a GLP-compliant 14-week dietary study, spirodiclofen (purity 98.6%) was administered to groups of Beagle dogs (four of each sex per dose) in the diet (mixed with water to form a paste) at dose levels of 0, 200, 630 or 2000 ppm (equal to 0, 7.7, 26.6 and 84.7 mg/kg bw per day for males and 0, 8.4, 28.0 and 81.0 mg/kg bw per day for females, respectively) for 14 weeks. Clinical observations were conducted twice daily, food and water intake were assessed daily and body weights were recorded weekly. Ophthalmoscopic and reflex assessments, body temperature and pulse recordings as well as electrocardiogram measurements were conducted before the start of the study (week −2) and at weeks 6 and 13. Blood was collected for haematological and clinical chemistry investigations at pretest (weeks −3 and −1) and during weeks 2, 6 and 13. Levels of plasma T_3 and T_4 and thyroxine-binding capacity were included in these investigations. Urinalysis was conducted at weeks −1, 2, 6 and 13. Gross necropsy (including organ weight assessments) and microscopic examinations were carried out at study termination. In addition, triglyceride levels

Table 11. Selected clinical chemistry findings[a] in the dog

Clinical chemistry parameter	Dietary concentration (ppm)				Historical control range[b]
	0	200	630	2000	
Male (*n* = 4)					
Cholesterol (mmol/l)					
Week −3	3.4 ± 0.5	2.3 ± 0.6	2.9 ± 0.6	3.3 ± 0.7	2.8–4.0
Week 2	3.1 ± 0.5	2.2 ± 0.4	2.9 ± 0.7	2.2 ± 0.5	2.8–3.5
Week 6	3.2 ± 0.4	2.3 ± 0.6	2.4 ± 0.8	1.9 ±0.3	3.2–4.2
Week 13	3.9 ± 0.9	2.8 ± 0.5	2.6 ± 0.5	2.6 ± 0.9	3.2–4.7
T_4 (nmol/l)					
Week −3	29 ± 6.6	24 ± 4.5	30 ± 4.6	28 ± 1.5	19–23
Week 2	29 ± 1.8	25 ± 3.6	26 ± 9.0	24 ± 4.4	19–23
Week 6	32 ± 3.6	28 ± 3.1	25 ± 6.5	19 ± 2.4	19–22
Week 13	27 ± 4.2	26 ± 2.1	24 ± 7.0	16 ± 2.5	19–24
Female (*n* = 4)					
Cholesterol (mmol/l)					
Week −3	2.4 ± 0.4	2.4 ± 0.3	2.7 ± 0.5	3.0 ± 0.8	3.0–4.2
Week 2	2.6 ± 0.4	2.3 ± 0.4	2.4 ± 0.3	2.1 ± 1.0	3.1–3.2
Week 6	2.7 ± 0.3	2.5 ± 0.3	2.4 ± 0.5	1.8 ± 0.5	3.4–4.2
Week 13	2.9 ± 0.9	2.6 ± 0.3	3.1 ± 0.6	1.9 ± 0.5	3.5–4.3
T_4 (nmol/l)					
Week −3	37 ± 5.9	32 ± 3.1	32 ± 9.0	33 ± 10.1	21–25
Week 2	31 ± 1.6	29 ± 2.1	23 ± 5.4	21 ± 4.9	21
Week 6	30 ± 2.8	32 ± 2.2	25 ± 1.5	19 ± 4.0	20–22
Week 13	28 ± 1.4	28 ± 2.1	24 ± 6.2	18 ± 5.1	21–24

From Wetzig & Hartmann (2001b, 2002a)

[a] Mean ± standard deviation.

[b] Historical control data for cholesterol and T_4 collected from three subchronic dog studies (n = 4 for each sex) conducted between 1993 and 1995; time periods for blood collection in these studies were prestudy, 2–4 weeks, 5–7 weeks and 13 weeks.

and activities of the following enzymes were determined in liver tissue: microsomal liver enzymes (N-DEM and O-DEM), cytochrome P450, cytochrome P450–dependent mono-oxygenases (ECOD, EROD, ALD), EH and the conjugation enzymes (GST, GLUT).

There were no compound-related effects on mortality, clinical signs, food and water consumption, haematology, urinalysis or ophthalmoscopic examinations. There was a dose-dependent decrease in body weight noted in both sexes, which was considered treatment related at and above the middle dose. Increased plasma aminotransferase (ASAT, ALAT), AP and GLDH activities were observed at doses of 630 ppm and above in females and at 2000 ppm in males. A trend of lower cholesterol and T_4 levels was observed at 630 and 2000 ppm (both sexes), as well as for albumin levels at 2000 ppm. When compared with historical control data (from 1996–1997), all of these parameters were within two standard deviations of the reference ranges (Table 11). There was an induction of phase I enzymes (cytochrome P450–dependent mono-oxygenases [ECOD and ALD]) in response to spirodiclofen administration at all doses in males and at the high dose in females. A slight increase was also measured for cytochrome P450 content in the 630 ppm and 2000 ppm groups. There were no effects on the phase II enzyme activities (GST and GLUT) or on triglyceride levels in the liver. EH was induced at the high dose in females only. Dose-dependent increases in N-DEM and O-DEM levels were seen in both sexes. The changes in liver enzyme activities suggest an induction of hepatic metabolic activity in response to administration of spirodiclofen. These changes were considered adaptive

Table 12. Incidences of selected microscopic findings in the dog

Histopathological finding	Incidence (no. of animals with finding)			
	Dietary concentration (ppm)			
	0	200	630	2000
Males (*n* = 4)				
Adrenal – cytoplasmic vacuolation	0	0	3	4
Adrenal – mononuclear cell infiltrate	0	1	1	4
Kidney – dilatation of proximal tubule	1	0	1	3
Epididymides – aspermia	0	0	1	2
Epididymides – oligospermia	0	0	2	0
Testes – Leydig cell vacuolation	0	0	2	4
Testes – Leydig cell hypertrophy	0	0	2	3
Testes – degeneration, diffuse	0	0	0	2
Prostate – immature	0	0	1	4
Thymus – atrophy	0	0	1	2
Females (*n* = 4)				
Adrenal – cytoplasmic vacuolation	0	2	3	4
Adrenal – mononuclear cell infiltrate	0	2	0	4
Kidney – dilatation of proximal tubule	0	1	0	2
Liver – periportal single-cell necrosis	0	0	0	1
Liver – cytoplasmic change	0	0	0	3
Liver – inflammatory infiltrates	0	0	0	4
Thymus – atrophy	0	0	0	1

From Wetzig & Hartmann (2001b, 2002a)

and thus not regarded as adverse. Dose-dependent increases in relative organ weights were observed in both sexes in the liver, pituitary and adrenal gland at 630 and 2000 ppm and in the kidney at 2000 ppm. Increased adrenal weights were also observed in two of four females at the low dose (200 ppm). Decreased prostate and thymus weights were also observed in males at 630 and 2000 ppm.

Histopathological examination revealed treatment-related findings in the liver, kidney, adrenal gland, prostate, epididymis, testes and thymus. Hepatocellular cytoplasmic changes, inflammatory infiltrates and single-cell necrosis were seen in females at the highest dose. Inflammatory infiltrates and necroses were considered to represent a toxic injury to the liver. Dilatation of the proximal tubules of the renal cortex was seen in both sexes at 2000 ppm. This finding was considered an adaptive effect, as no degenerative changes were noted in the kidney. In the testes, vacuolation and hypertrophy/activation of Leydig cells were observed at 630 and 2000 ppm. In addition, degeneration and/or immaturity of the testicular germinal epithelium, as well as oligospermia and aspermia of the epididymides, were detected at doses of 630 ppm and above. The prostates of all high-dose males and one male of the intermediate dose group were completely undeveloped compared with those of control males of similar age. Mild atrophy of the thymic cortex was observed in one male at 630 ppm and in both sexes at 2000 ppm. This finding was considered more likely due to an indirect toxicity rather than to direct cytotoxicity to thymic lymphocytes. A dose-dependent increase in cytoplasmic vacuolation of the adrenal cortex was observed in females at all doses and in males at 630 ppm and above, which was interpreted as a morphological correlate to effects on lipid and steroid metabolism (Table 12).

The NOAEL for males in this study was 200 ppm (equal to 7.7 mg/kg bw per day), based on decreased body weight gain, decreased prostate and thymus weights, increased relative liver, pituitary and adrenal weights and histopathological findings in the adrenal gland, thymus, epididymides, testes and prostate at 630 ppm. A NOAEL could not be established for females in this study, as adrenal effects were noted in females at all doses tested; the LOAEL for females was therefore 200 ppm, equal to 8.4 mg/kg bw per day (Wetzig & Hartmann, 2001b, 2002a).

In a GLP-compliant 1-year dog study, spirodiclofen (purity 97.8%) was administered to groups of Beagle dogs (four of each sex per group) in the diet (mixed with water to form a paste) at dose levels of 0, 20, 50, 150 or 500/600 (equal to 0, 0.56, 1.38, 4.33 and 16.1 mg/kg bw per day for males and 0, 0.59, 1.52, 4.74 and 17.7 mg/kg bw per day for females, respectively) for 1 year. The highest dose was increased from 500 to 600 ppm in week 4 of the study. Clinical observations were conducted twice daily, food and water intake were assessed daily and body weights were recorded weekly. Ophthalmoscopic and reflex assessments, body temperature recordings, as well as electrocardiogram and blood pressure measurements were conducted before the start of the study (week −3) and at weeks 6, 12, 26/27, 39 and 52. Blood was collected for haematological and clinical chemistry investigations at pretest (weeks −3 and −1) and during weeks 3, 6, 12, 26, 39 and 52. Levels of plasma T_3 and T_4 and thyroxine-binding capacity were included in these investigations. Additional blood samples were taken from high-dose animals during week 20 in order to determine the plasma concentration of spirodiclofen and its metabolite, BAJ 2510, at 0, 2, 4, 7 and 24 h following dosing. Urinalysis was conducted at the same time points as blood collections. Gross necropsy (including organ weight assessments) and microscopic examinations were carried out at study termination. Additional urine samples were collected at week 28 from some high-dose and control animals in order to determine concentrations of the BAJ 2510 metabolite. In addition, triglyceride levels and activities of the following enzymes were determined in liver tissue: microsomal liver enzymes (N-DEM and O-DEM), cytochrome P450, cytochrome P450–dependent mono-oxygenases (ECOD, EROD, ALD), EH and the conjugation enzymes (GST, GLUT).

There were no compound-related effects on mortality, clinical signs, food and water consumption, body weight gain, urinalysis, haematology or ophthalmoscopic examinations. Cholesterol levels were decreased slightly in the 150 and 600 ppm groups; however, values were found to be within historical control ranges (Table 13). No other findings were noted in the clinical chemistry investigations. Increases in N-DEM and O-DEM activities in liver tissue were noted. These increases were dose dependent for N-DEM, beginning at 50 ppm, and noted at the high dose only for O-DEM; they were indicative of an induction of hepatic metabolic activity in response to spirodiclofen administration, which was not considered to be adverse. Dose-related increases in relative weight were observed in adrenal glands of both sexes (more pronounced in females), as well as in testes and prostates in males (Table 14). Histopathological examinations revealed an increased incidence of cortical vacuolation in the zona fasciculata of the adrenal gland in both sexes at and above 150 ppm. In the testes, an increased incidence of Leydig cell vacuolation was noted at the high dose. Slight Leydig cell hypertrophy and tubular degeneration were each observed in one male at 500/600 ppm (Table 15). There were no histopathological findings correlating with the increased prostate weights.

The NOAEL is 50 ppm, equal to 1.38 mg/kg bw per day and 1.52 mg/kg bw per day for males and females, respectively, based on increased relative adrenal weights in both sexes, increased relative testis weight in males and histopathological findings in the adrenal gland of both sexes at 150 ppm (Wetzig & Ruehl-Fehlert, 2001; Wetzig & Hartmann, 2002b).

Table 13. Cholesterol findings in the dog (1-year study)

Week	Cholesterol level[a] (mmol/l)					
	Dietary concentration (ppm)					Historical control data[b]
	0	20	50	150	500/600	
Males (*n* = 4)						
Week −3	3.93 ± 0.78	4.62 ± 0.70	4.84 ± 0.54	4.81 ± 0.44	4.70 ± 0.89	3.93 ± 0.78
Week 3	3.29 ± 0.47	3.75 ± 0.64	3.79 ± 0.76	3.73 ± 0.46	3.52 ± 0.90	3.16 ± 0.27
Week 6	3.16 ± 0.27	3.13 ± 0.49	3.62 ± 0.77	3.06 ± 0.34	2.32 ± 0.59	2.75 ± 0.71
Week 12	2.75 ± 0.71	3.30 ± 0.47	3.90 ± 0.76	3.78 ± 0.27	2.92 ± 0.66	3.26 ± 0.62
Week 26	3.26 ± 0.62	4.26 ± 0.51	4.07 ± 0.91	4.27 ± 0.59	3.50 ± 0.52	3.55 ± 0.73
Week 52	3.28 ± 0.57	4.82 ± 0.99	4.09 ± 1.05	3.88 ± 0.33	3.77 ± 0.31	3.28 ± 0.57
Females (*n* = 4)						
Week −3	3.93 ± 0.66	3.76 ± 0.44	4.09 ± 0.74	4.12 ± 0.80	3.99 ± 0.25	3.93 ± 0.66
Week 3	3.88 ± 0.68	3.18 ± 0.32	3.26 ± 0.59	3.05 ± 0.36	3.07 ± 0.42	3.16 ± 0.77
Week 6	3.16 ± 0.77	2.77 ± 0.18	2.73 ± 0.33	2.82 ± 0.32	2.67 ± 0.64	2.73 ± 0.29
Week 12	2.73 ± 0.29	3.33 ± 0.39	3.08 ± 0.45	2.87 ± 0.31	3.04 ± 0.48	4.39 ± 0.84
Week 26	4.39 ± 0.84	4.81 ± 1.01	3.88 ± 0.75	3.47 ± 0.89	4.21 ± 0.59	5.90 ± 1.56
Week 52	5.89 ± 1.71	4.58 ± 0.69	4.09 ± 0.02	5.54 ± 2.36	4.77 ± 1.36	5.89 ± 1.71

From Wetzig & Ruehl-Fehlert (2001); Wetzig & Hartmann (2002b)

[a] Mean ± standard deviation.

[b] Historical control data collected from four chronic dog studies (*n* = 4 in each sex) conducted between 1994 and 1996; time periods for blood collection in these studies were prestudy, 4–6 weeks and weeks 13, 26, 39 and 52.

Table 14. Selected relative organ weight findings in the dog (1-year study)[a]

Organ	Dietary concentration (ppm)				
	0	20	50	150	500/600
Males (*n* = 4)					
Body weight (kg)	15.0 ± 2.9	14.8 ± 1.2	15.5 ± 0.67	15.1 ± 1.8	16.28 ± 2.20
Liver (g/kg)	29.0 ± 4.9	34.9 ± 5.8	35.8 ± 14.3	34.8 ± 3.8	34.24 ± 4.11
Adrenal (g/kg)	0.106 ± 0.024	0.121 ± 0.027	0.119 ± 0.028	0.115 ± 0.014	0.123 ± 0.027
Testis (g/kg)	1.38 ± 0.16	1.64 ± 0.20	1.4 ± 0.19	1.77 ± 0.13	1.79 ± 0.086
Prostate (g/kg)	0.70 ± 0.28	0.71 ± 0.17	0.72 ± 0.261	0.73 ± 0.25	0.83 ± 0.20
Females (*n* = 4)					
Body weight (kg)	14.2 ± 1.9	13.6 ± 1.7	13.9 ± 1.2	14.4 ± 1.1	14.7 ± 1.2
Liver (g/kg)	32.4 ± 1.5	30.1 ± 6.1	32.6 ± 8.8	30.5 ± 5.5	32.9 ± 4.0
Adrenal (g/kg)	0.131 ± 0.030	0.139 ± 0.021	0.130 ± 0.014	0.145 ± 0.014	0.154 ± 0.012
Kidney (g/kg)	4.2 ± 0.41	4.6 ± 0.54	4.8 ± 1.02	4.8 ± 0.50	5.0 ± 0.14

From Wetzig & Ruehl-Fehlert (2001); Wetzig & Hartmann (2002b)

[a] Organ weights are relative to body weight; mean ± standard deviation.

Table 15. Incidences of selected microscopic findings in the dog (1-year study)

Histopathological finding	Incidence (no. of animals with finding)				
	Dietary concentration (ppm)				
	0	20	50	150	500/600
Males (*n* = 4)					
Adrenal – cortical vacuolation	1	2	0	4	4
Kidney – increased tubular pigment	2	4	3	0	1
Thyroid – vacuoles	2	2	3	1	1
Testes – Leydig cell vacuolation	0	0	0	0	4
Testes – Leydig cell hypertrophy	0	0	0	0	1
Testes – tubular degeneration	0	0	0	0	1
Females (*n* = 4)					
Adrenal – cortical vacuolation	1	1	0	3	4
Kidney – increased tubular pigment	1	1	1	1	3
Thyroid – vacuoles	1	0	1	0	3

From Wetzig & Ruehl-Fehlert (2001); Wetzig & Hartmann (2002b)

(b) Dermal application

Rat

In a GLP-compliant 4-week dermal toxicity study, spirodiclofen (purity 97.9%) was applied (undiluted; moistened with water immediately prior to application) to the shaved skin of Wistar rats (five of each sex per dose) at dose levels of 0 or 1000 mg/kg bw per day (limit dose), 6 h/day, 5 days/week (total of 22 applications), during a 28-day period. Clinical observations (including examinations for signs of dermal irritation) were performed daily, and body weights and food consumption were determined weekly. Swelling of the skin was evaluated at regular intervals throughout the study by measuring skinfold thickness in the treatment area. Clinical laboratory examinations and gross necropsy (with organ weights and tissue sampling) were performed on all surviving animals at termination. Histopathological examination of the liver, lung, heart, spleen, thymus, kidneys, testes, epididymides, brain, adrenal glands and skin was performed. There were no compound-related effects on mortality, clinical signs, body weight, food consumption, haematology, clinical chemistry, organ weights or gross and histological pathology.

Based on the study results, the NOAEL was 1000 mg/kg bw per day (the limit dose) for males and females (Kroetlinger & Sander, 1999). It was noted that several tissues were not weighed or subjected to histopathological assessment, including the prostate and all female reproductive tissues, among others.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a GLP-compliant carcinogenicity study, spirodiclofen (purity 97.6–98.6%) was administered to groups of CD-1 mice (50 animals of each sex per dose) in the diet (in an acetone/corn oil mixture) at concentrations of 0, 25, 3500 or 7000 ppm (equal to 0, 4.1, 610 and 1216 mg/kg bw per day for

males and 0, 5.1, 722 and 1495 mg/kg bw per day for females, respectively) for 18 months. Body weight and food consumption determinations were conducted weekly for approximately 13 weeks and once a month thereafter; detailed clinical examinations of each animal were conducted weekly throughout the study. Standard haematological and differential leukocyte parameters were evaluated at approximately 12 and 18 months into the study. All animals were subjected to a postmortem examination, which included documenting and saving all gross lesions, weighing designated organs and collecting tissues for histopathological evaluation.

There were no compound-related effects on mortality, clinical signs, body weight, food consumption or haematological examinations. Increased absolute and relative liver weights were observed in males at 3500 ppm and in both sexes at 7000 ppm. Increased absolute and relative adrenal gland weights were noted at 3500 and 7000 ppm in both sexes. Increased testis weights (absolute and relative) were observed in males at 3500 and 7000 ppm. Decreased absolute and relative kidney weights were observed at 3500 and 7000 ppm in both sexes. Gross pathology showed enlarged adrenal glands at 3500 and 7000 ppm in both sexes. Additional findings that were noted included discoloured testis in mid- and high-dose males (0, 0, 3, 7) and increased incidence of focal eye opacity in high-dose males (males: 1, 0, 0, 6; females: 1, 3, 0, 0). Histopathological examination revealed increases in the incidence and severity of vacuolation in the adrenal cortex at 3500 and 7000 ppm in both sexes. The Meeting noted that the incidence of vacuolation of the adrenal cortex was also increased in low-dose females and that the incidence of lymphocytic infiltrate was increased in the adrenals of high-dose females (males: 0, 0, 0, 1; females: 0, 0, 2, 5*; * $P \leq 0.05$). Adrenal gland pigmentation was noted in females at all dose levels and in males at 7000 ppm only. The study authors noted that the incidence in low-dose females was within historical control ranges (reportedly up to 37%, based on two studies) and was not considered to be clearly related to treatment at this dose level. Although the incidence of pigmentation was only slightly above the reported historical control range and the increase in vacuolation was not statistically significant for low-dose females, as the adrenal gland is clearly a target organ, the Meeting concluded that these findings were toxicologically relevant and represent a marginal LOAEL for females at this dose level. In males, dose-dependent increases in the incidence and severity of hepatocytomegaly were observed, and increases in the incidence and severity of hypertrophy and hyperplasia of the interstitial cell in the testes were noted at 3500 and 7000 ppm. The lesion in the testis consisted of greatly increased cell size as well as numbers of cells. Other findings noted in male reproductive tissues of high-dose animals included an increase in testicular degeneration and epididymal aspermia. Mineralization in the brain was significantly increased over control in 7000 ppm females. However, as there was no significant change in males and no dose–response relationship for the lesion in either sex, this finding was considered to be a chance occurrence and not treatment related (Table 16). A dose-related increase in the incidence of amyloid was observed in various organs of both sexes. There were no histopathological findings that correlated with the decreased kidney weights.

Neoplastic lesions were noted in the liver in male and female mice. Increased incidences of hepatocellular adenomas and carcinomas were observed at doses of 3500 ppm and above (Table 17). In comparison with historical control data, the combined frequencies of hepatocellular adenoma and carcinoma at 3500 ppm (16% in males and 10% in females) and 7000 ppm (22% in males and 6% in females) were higher than the ranges seen in either in-house control (4–14% in males and 0–2% in females) or literature historical data presented in the study report (0–9.6% in males and 0–2.7% in females). The Meeting noted that although preneoplastic lesions were not observed at doses lower than those at which the liver tumours were observed, this may be due to the large dose spacing. Additionally, it is noted that these tumours were observed only at high doses (≥3500 ppm), which also produced hepatotoxicity, and are likely to exhibit a threshold in their dose–response. The NOAEL for carcinogenicity was 25 ppm (equal to 4.1 mg/kg bw per day for males and 5.1 mg/kg bw per day for females) based on the increased incidence of hepatocellular adenoma and carcinoma at 3500 ppm.

Table 16. Incidences of selected non-neoplastic microscopic findings in the mouse

Histopathological finding	Incidence (no. of animals with finding)			
	Dietary concentration (ppm)			
	0	25	3500	7000
Males (*n* = 50)				
Adrenal pigmentation (corticomedullary junction)	7	5	11	27*
Adrenal vacuolation (cortex)	0	0	31*	37*
Liver hepatocytomegaly	2	6	17*	21*
Heart – atrial thrombus	0	1	1	9*
Testes – tubular degeneration	18	18	22	28
Testes hypertrophy and/or hyperplasia of interstitial cells	6	6	26*	31*
Epididymides aspermia	15	15	15	26*
Brain – mineralization	12	4	12	8
Females (*n* = 50)				
Adrenal pigmentation (corticomedullary junction)	11	20*	45*	42*
Adrenal vacuolation (cortex)	1	6	49*	48*
Brain – mineralization	5	7	4	14*

From Wahle (2000)
* $P < 0.05$

Table 17. Liver tumour incidences in the mouse

Tumour type	Incidence (no. of animals with tumour) / % of total			
	Dietary concentration (ppm)			
	0	25	3500	7000
Males (*n* = 50)				
Hepatocellular adenoma	0	0	5 / 10*	6 / 12*
Hepatocellular carcinoma	1 / 2	1 / 2	3 / 6	5 / 10
Hepatocellular adenoma and carcinoma combined	1 / 2	1 / 2	8 / 16*	10 / 20*[a]
Females (*n* = 50)				
Hepatocellular adenoma	0	0	3 / 6	1 / 2
Hepatocellular carcinoma	0	0	2 / 4	2 / 4
Hepatocellular adenoma and carcinoma combined	0	0	5 / 10*	3 / 6

From Wahle (2000)
* $P < 0.05$
[a] One male had both types of tumours.

The study authors identified 25 ppm (equal to 4.1 mg/kg bw per day for males and 5.1 mg/kg bw per day for females) as the NOAEL for systemic toxicity based on increased liver weights, decreased kidney weights, enlarged adrenal glands and histopathological findings in the adrenal gland in both sexes and the following findings in males: increased liver and testes weights and histopathological findings in the testes and liver at 3500 ppm. However, as the Meeting considered the increased adrenal findings in females at the low dose to be toxicologically relevant, a marginal LOAEL for females

was identified at 25 ppm (equal to 5.1 mg/kg bw per day), and thus a NOAEL for systemic toxicity in females was not identified (Wahle, 2000).

Rats

In a GLP-compliant combined chronic toxicity and carcinogenicity study, spirodiclofen (purity 97.6–98.6%) was administered to groups of Wistar Hsd Cpb:WU rats (50 of each sex per dose) via the diet (mixed with 1% peanut oil) at dose levels of 0, 50, 100, 350 or 2500 ppm (equal to 0, 2.0, 4.1, 14.7 and 110.1 mg/kg bw per day for males and 0, 2.9, 5.9, 19.9 and 152.9 mg/kg bw per day for females, respectively) for 2 years. Additional groups of rats (10 of each sex per dose) were treated likewise with spirodiclofen for interim sacrifice after 1 year. Clinical observations were conducted twice daily, and body weights were recorded weekly for 13 weeks and then every 2 weeks thereafter. Food consumption was recorded twice weekly for 37 weeks and then every 4 weeks thereafter. Water consumption was recorded every 4 weeks throughout the study. Ophthalmological investigations were conducted on all animals before treatment, on control and high-dose animals before interim sacrifice (week 50) and on all animals at the end of treatment (week 102). Samples for clinical chemistry, urinalysis and haematology were collected from animals (10 of each sex per dose) at four time points throughout the study (at approximately weeks 27, 53, 79 and 105). Measurements of thyroid hormones were included in the clinical chemistry investigations. A functional observational battery (FOB) was conducted on 10 rats of each sex per dose at week 77. Gross necropsy and histopathological examinations as well as recording of organ weights were carried out at interim and terminal sacrifices.

There were no treatment-related clinical signs throughout the study. Mortality was increased in 2500 ppm females (incidence of deaths prior to scheduled sacrifice: 21, 18, 19, 15, 24; but with no statistical significance), whereas mortality of 2500 ppm males was slightly decreased (incidence of deaths prior to scheduled sacrifice: 19, 20, 14, 19, 9). Spirodiclofen had no effect on water consumption. At the highest dose, body weight–related food consumption was slightly increased in both sexes. Significant decreases in body weights were observed in both sexes in the 2500 ppm group (decreased by 8–10% for males up to week 101; by 6–7% for females up to week 53). Body weight gains were decreased at 2500 ppm in both sexes up to week 3 and recovered thereafter. The FOB performed in study week 77, the home cage observations and observations during handling did not reveal evidence of a neurotoxic potential. A slightly higher incidence of post-capsular lens opacity in males dosed with 350 and 2500 ppm was noted (22%, 24%, 25%, 39% and 32%, respectively). In females, this finding was distributed evenly across control and treatment groups. Owing to a lack of a clear dose–response relationship and histopathological correlates, combined with the appearance of the effect in one sex only, a clear relationship with treatment was not established for the lens opacity. There were no significant effects on haematological or urinalysis parameters assessed; however, significant increases in AP and decreases in cholesterol and triglyceride levels (not statistically significant) were observed in both sexes at 2500 ppm at all test points (Table 18). Significantly increased T_4 levels were observed in 2500 ppm males at weeks 53 and 105. Increased TSH levels were observed at 2500 ppm in both sexes, but the statistical significance was observed only in females at weeks 79 and 105. There was no clear dose–response relationship or consistency over time for the thyroid hormone findings, and thus a relationship with treatment was not clearly established (Table 19).

At the interim sacrifice, an increased incidence of adrenal cortex vacuolation (microvesicular and/or large vacuoles) in zona fasciculata cells was observed in high-dose males; minimal to slight diffuse adrenocorticocellular hypertrophy was also observed in three high-dose males. In high-dose females, an increased portion of ovarian stroma and slight, possibly spurious, changes in estrous cycle were observed. These changes were characterized as a decrease in the number of females in metestrus/diestrus, with a corresponding increase in the number of those in estrus (based on morphology of the vaginal epithelium). At final necropsy, increased incidences of liver discoloration

Table 18. Selected clinical chemistry findings in the rat

Dietary concentration (ppm)	Week	AP (U/l)	Cholesterol (mmol/l)	Triglyceride (mmol/l)	AP (U/l)	Cholesterol (mmol/l)	Triglyceride (mmol/l)
		Males			Females		
0	27	175	4.44	4.31	122	2.17	2.00
50		196	2.72	1.91	128	2.46	2.13
100		186	2.20	2.18	134	2.04	1.82
350		201	2.57	1.75	150	2.21	1.66
2500		305**	1.90	1.36	226**	2.07	1.16*
0	53	203	3.15	1.73	123	2.32	1.69
50		199	3.90	2.13	112	2.79	2.38
100		180	2.91	2.10	115	2.54	1.89
350		213	3.81	2.09	135	2.11	1.82
2500		279**	2.78	1.81	183	2.06	1.31
0	79	171	3.59	2.70	122	2.25	2.12
50		178	4.25	2.45	100	3.71	6.30
100		163	3.07	2.39	105	2.47	2.73
350		192	4.44	2.42	120	2.24	2.06
2500		277**	2.89	1.84	226**	2.06	1.91
0	105	176	4.11	2.39	117	2.59	2.26
50		167	4.41	2.16	110	2.74	2.66
100		212	3.88	2.42	112	2.77	2.70
350		197	3.77	2.15	135	2.17	2.43
2500		265**	3.29	1.57	188*	2.21	2.06

From Wirnitzer, Bach & Hartmann (2000); Wirnitzer & Hartmann (2002b)
* $P < 0.05$; ** $P < 0.01$

Table 19. Thyroid hormone findings in the rat

Clinical chemistry parameter	Dietary concentration (ppm)				
	0	50	100	350	2500
Males (*n* = 10)					
T_4 (nmol/l)					
- Week 27	45	51	51	53	55
- Week 53	44	46	48	52*	52*
- Week 79	51	50	52	51	57
- Week 105	48	45	48	52	56*
TSH (µg/l)					
- Week 27	3.30	2.26	3.04	3.05	2.88
- Week 53	2.04	1.81	3.03	2.38	3.04
- Week 79	2.67	1.30	3.27	2.98	3.32
- Week 105	1.99	1.00**	2.09	2.15	2.68
Females (*n* = 10)					
T_4 (nmol/l)					
- Week 27	32	31	33	39	35
- Week 53	39	35	34	34	34
- Week 79	40	37	38	44	41

Table 19 (contd)

Clinical chemistry parameter	Dietary concentration (ppm)				
	0	50	100	350	2500
- Week 105	40	42	41	43	44
TSH (µg/l)					
- Week 27	1.93	1.56	1.40	1.80	1.89
- Week 53	0.97	1.30	1.19	0.99	1.48
- Week 79	1.10	1.45	1.41	1.43	2.48*
- Week 105	1.22	1.26	1.47	1.13	2.55**

From Wirnitzer, Bach & Hartmann (2000); Wirnitzer & Hartmann (2002b)
* $P < 0.05$; ** $P < 0.01$

Table 20. Incidences of selected non-neoplastic microscopic and macroscopic findings in the rat at terminal sacrifice

Histopathological finding	Incidence (no. of animals with finding)				
	Dietary concentration (ppm)				
	0	50	100	350	2500
Males (*n* = 50)					
Adrenal vacuolation/hypertrophy	3	1	5	5	25*
Thyroid – colloidal alteration	23	23	28	28	35*
Jejunum – vacuolated enterocytes	3	0	4	3	18**
Nasal cavity – atrophy/degeneration of olfactory epithelium	13	19	15	16	25
Liver – tigroid basophilic foci	12	14	14	10	21*
Females (*n* = 50)					
Jejunum – vacuolated enterocytes	5	0[a]	0	1	17**
Uterus – nodules[b]	8	7	9	9	17

From Wirnitzer, Bach & Hartmann (2000); Wirnitzer & Hartmann (2002b)
* $P < 0.05$; ** $P < 0.01$
[a] $n = 49$.
[b] Macroscopic finding.

(females), dilatation of renal pelvis (females), consistency changes of pancreas (females, only intercurrent deaths) and uterine nodules were seen at the highest dose of 2500 ppm. Terminal histopathological examinations also revealed findings at the high dose. These included an increased incidence of vacuolated enterocytes in the jejunum in both sexes and an increased incidence and severity of vacuolation in zona fasciculata cells of the adrenal cortex, increased adrenocorticocellular hypertrophy, an increased incidence of colloidal alteration in the thyroid, tigroid basophilic foci in the liver and atrophy/degeneration of the olfactory epithelium in the nasal cavity in males (Table 20). The pathology report noted several incidences of age-dependent non-neoplastic findings that exhibited a treatment-related decrease in males and/or females of the high dose group. These findings were as follows: arteritis/periarteritis in the blood vessels of various organs in males, chronic progressive nephropathy in the kidneys of males and females, diffuse transitional cell hyperplasia in the renal pelvis of both sexes, mineralization in the kidneys of females, squamous cell hyperplasia of the uterine cervix in females, peliosis in the adrenal cortex in females, focal hyperplasia in the adrenal medulla in females, pigment deposits in the spleen and mesenteric lymph node in both sexes, and epithelial hyperplasia in the thymus of both sexes.

Table 21. Incidences of neoplastic lesions in the rat

Neoplastic lesion	Incidence (no. of animals with lesions) / % of total					Historical control range[b] (%)
	Dietary concentration (ppm)					
	0	50	100	350	2500	
Males (*n* = 50)						
Testes – Leydig cell adenoma	2 / 4	1 / 2	0 / 0	4 / 8	10 / 20*	2–8
Testes – Leydig cell hyperplasia	4 / 8	4 / 8	4 / 8	7 / 14	19 / 38**	2–10
Thyroid – C-cell adenoma	4 / 8	6 / 12	5 / 10	4 / 8	7 / 14	6–24
Females (*n* = 50)						
Uterus – adenocarcinoma[a]	4 / 8	5 / 10	3 / 6	2 / 4	14 / 28**	2–10
Metastases of uterine origin						
- Ovary	1	0	0	1	5	
- Lung	0	1	1	1	5*	
- Kidney	1	1	2	0	6	
- Pancreas	1	1	1	1	5	
- Liver	0	0	2	1	5*	
- Spleen	0	1	1	0	3	
- Mesenteric lymph node	0	0	0	0	3	
- Bone marrow	0	0	0	0	2	
Thyroid – C-cell adenoma	2 / 4	2 / 4	3 / 6	5 / 10	6 / 12	6–22

From Wirnitzer, Bach & Hartmann (2000); Wirnitzer & Hartmann (2002b)
* $P < 0.05$; ** $P < 0.01$
[a] Incidence includes decedents.
[b] Historical control range from six 24- to 25-month studies conducted in the same laboratory between 1994 and 1996.

An increased incidence of treatment-related neoplastic findings in reproductive organs of males (testes) and females (uterus) was observed (Table 21). In males, a significantly increased frequency of Leydig cell adenomas was observed concurrently with an increased incidence of Leydig cell hyperplasia at the high dose. The average severity of the hyperplasia was also slightly increased at the high dose. The majority of Leydig cell adenomas and focal hyperplasias were found in males at the termination of the study, suggesting a late onset of these alterations. In females, an increased incidence of uterine adenocarcinoma was observed at 2500 ppm. The majority of the uterine adenocarcinomas (11 out of 14) were found in females that died or had to be sacrificed before the termination of the study. The pathology report also indicated that many of the adenocarcinomas had metastasized by invasion and intra-abdominal spread into various organs of the abdominal cavity, such as ovaries, liver, spleen, pancreas, mesenteric lymph node and kidney; metastases were also found in the lung and bone marrow. It was noted that the bone marrow was an uncommon location for metastases of uterine adenocarcinomas. Compared with historical control data (provided in an amendment to the main study), the incidence of Leydig cell adenomas in high-dose males (20%) exceeded the historical control range (2–8%), whereas the incidence of Leydig cell hyperplasia exceeded the historical control range (2–10%) at the middle (14%) and high (38%) doses. The Meeting considered the increase in Leydig cell hyperplasia to be treatment related at both the middle and high doses, based on the exceedance of the historical control range at these doses. The incidence of uterine adenocarcinoma also exceeded the historical control range (2–10%) at the high dose. It was suggested by the study authors that the observed increases in tumours in males and females were not indicative of a primary carcinogenic effect, but rather secondary to interference of spirodiclofen with steroid biosynthesis.

The NOAEL for oncogenicity is 350 ppm, equal to 14.7 mg/kg bw per day for males and 19.9 mg/kg bw per day for females, based on an increased incidence of Leydig cell adenomas and uterine adenocarcinomas at 2500 ppm. The study authors concluded that the NOAEL for systemic toxicity was 350 ppm, based on decreased body weights, alterations in clinical chemistry parameters (increased AP and decreased cholesterol and triglyceride levels) and histopathological findings in the jejunum, adrenal gland, thyroid, uterus, liver and nasal cavity at 2500 ppm. The Meeting identified the NOAEL for systemic toxicity in males to be 100 ppm, equal to 4.1 mg/kg bw per day, based on the increased incidence of Leydig cell hyperplasia at 350 ppm (Wirnitzer, Bach & Hartmann, 2000; Wirnitzer & Hartmann, 2002b).

2.4 Genotoxicity

A battery of mutagenicity studies with spirodiclofen was conducted to assess the potential for inducing gene mutation or chromosomal aberrations in vitro, as well as its clastogenic potential in vivo. The study results (summarized in Table 22) were negative. Spirodiclofen did not demonstrate any genotoxic potential under the conditions tested.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a GLP-compliant two-generation reproduction study, spirodiclofen (purity 98.6%) was administered to groups of Crl:WI(WU)BR Wistar rats (25 of each sex per dose; for both F_0 and F_1 generations) for 12–13 weeks prior to mating, as well as during mating, gestation and lactation. Spirodiclofen was administered via the diet at doses of 0, 70, 350 or 1750 ppm (equal to 0, 5.2, 26.2 and 134.8 mg/kg bw per day for F_0 males; 0, 5.5, 27.6 and 139.2 mg/kg bw per day for F_0 females; 0, 6.4, 30.2 and 177.6 mg/kg bw per day for F_1 males; 0, 7.0, 34.4 and 192.7 mg/kg bw per day for F_1 females). Clinical signs, body weights, food intake, mating performance, fertility, duration of pregnancy, estrous cycling and sperm parameters (sperm motility, morphology and counts) were examined in F_0 and F_1 rats. Clinical chemistry parameters were assessed in F_1 adults (10 of each sex per dose) just prior to mating. Litter size, ratio of males to females, clinical signs, pup weight at birth as well as viability, lactation and body weight gain were studied in F_1 and F_2 offspring. Developmental milestones (preputial separation and vaginal opening) were examined in F_1 weanlings. Selected clinical chemistry parameters were examined in F_1 parent animals. Necropsies were done in all rats. Selected organs were weighed (from F_0 and F_1 adults as well as F_1 and F_2 weanlings), and histopathological evaluations were performed on selected organs of F_0 and F_1 rats.

No treatment-related clinical signs or deaths occurred in parental male or female rats of either generation receiving any dose of the test material. Mean body weights were significantly decreased throughout the study in high-dose F_0 males (by 6–8%) and at various time points in mid-dose F_0 males (by 5%). Mean body weights were significantly decreased in high-dose F_1 males (by 17–23%) throughout the study, whereas low- and mid-dose F_1 males had weights similar to those of controls. Body weight gain was also decreased for high-dose F_0 and F_1 males (by 9% and 16%, respectively) over the entire study. In F_0 females, decreases in body weight were observed at the high dose during a few of the premating weeks (by 5–7%; overall gain by 19%) and during gestation and lactation (by 5–10%). Overall body weight gain during premating was also decreased at the middle dose (by 11%) in F_0 females. High-dose F_1 females had significantly decreased body weights during lactation (by 7%), and overall body weight gain during gestation was also decreased (by 12%). Food consumption

Table 22. Results of genotoxicity studies on spirodiclofen

End-point	Test object	Concentration	Purity (%)	Results	GLP compliant	Reference
In vitro						
Reverse mutation[a,b]	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537	0–5000 µg/plate	99.1	Negative	Yes	Herbold (1996a)
Forward mutation[a,c,d,e]	Chinese hamster lung cells (V79), *Hgprt* locus	4–20 µg/ml without S9; 10–80 µg/ml with S9	99.1	Negative	Yes	Brendler-Schwaab (1997)
Chromosomal aberration[a,f,g,h]	Chinese hamster lung cells (V79)	Trial 1: 0–80 µg/ml without S9; 0–160 µg/ml with S9 Trial 2: 0–12 µg/ml without S9	99.1	Negative	Yes	Herbold (1996b)
In vivo						
Micronucleus[i,j]	Mouse (NMRI, 5 of each sex per dose) bone marrow	800 mg/kg bw (intraperitoneal) in 0.5% aqueous Cremophor	99.1	Negative	Yes	Herbold (1996c)

S9, 9000 × *g* rat liver supernatant

[a] Positive and negative controls included; conducted with and without exogenous metabolic activation.

[b] Tested in triplicate, using both the standard plate method and the preincubation method.

[c] Two trials conducted; tested in duplicate in each trial.

[d] Cytotoxicity was observed at ≥15 µg/ml without S9 and at 80 µg/ml with S9; compound insolubility was reported at final concentrations of ≥156 µg/ml.

[e] In Trial 1 with metabolic activation, there was an increase in mutation frequency at all dose levels. The increases were not dose responsive, were not reproduced in Trial 2 and were all within the historical control range; therefore, it was concluded that these increases were not indicative of a mutagenic response.

[f] Two trials were conducted.

[g] Without S9 mix, cytotoxic effects were observed at 0.75 µg/ml and above. With S9 mix, cytotoxic effects were observed at 20 µg/ml and above. Doses were lowered for Trial 2 (without S9 mix) due to the severity of cytotoxicity observed at the concentrations used in Trial 1. Compound insolubility was reported at ≥100 µg/ml.

[h] Statistically significant increases in metaphase aberrations were noted at 3 µg/ml (18 h harvest) without S9 and at 80 µg/ml (30 h harvest) with S9; however, values were within historical control ranges, and therefore it was concluded that this increase did not represent a clastogenic response. Significant cytotoxicity (≥50% decrease in the mitotic index) was observed at these doses.

[i] Analysed five rats of each sex per group. Positive and negative controls were included. Clinical signs included apathy, roughened fur, spasm and eyelids stuck together. One mortality occurred.

[j] A decrease in the ratio of polychromatic to normochromatic erythrocytes was noted in treated groups, which may be indicative of bone marrow toxicity.

was not affected in the F_0 parental generation or in F_1 males. F_1 females at 1750 ppm had slightly increased food consumption. Clinical chemistry parameters were evaluated in a subset of F_1 males and females at the end of the premating period. Dose-responsive decreases in plasma triglyceride, cholesterol and unesterified fatty acid levels were observed in males and females, some of which attained statistical significance at all dose levels (Table 23). AP activity was elevated slightly more than 2-fold in high dose group F_1 male and female rats. Statistically significant decreases in absolute (decreased by 12–15%) and relative (decreased by 8–9%) liver weights were observed in F_0 males at all dose levels and in high-dose F_1 males (decreased by 26% and 13%, absolute and relative, respectively). However, based on the lack of dose dependence or statistically significant findings in the livers of females, these were not considered to be treatment related. Relative adrenal (both sexes) and testes weights were increased at 1750 ppm in F_0 animals. In high-dose F_1 males, a slight increase in absolute adrenal weight and a statistically significant increase in relative adrenal weight were observed. The absolute mean weights for the testes, epididymides, seminal vesicles and prostate were reduced in

Table 23. Selected clinical chemistry findings in F_1 adults

Parameter	Males				Females			
	Dietary concentration (ppm)							
	0	70	350	1750	0	70	350	1750
Cholesterol,[a] mmol/l (% change)	2.55	2.30	2.07** (−19)	1.58** (−38)	2.24	2.33	2.20	1.69 (−25)
Triglyceride,[b] mmol/l (% change)	2.62	1.84**	1.68** (−36)	0.96** (−63)	1.36	1.22	1.02	0.58** (−57)
Non-esterified fatty acid (mmol/l)	0.39	0.38	0.32	0.24**	0.54	0.47	0.44*	0.32**

From Eiben (2000, 2002, 2003, 2004)
* $P < 0.05$; ** $P < 0.01$
[a] Historical control values: male: 2.33 (±2 standard deviations [SD]: 1.52–3.15) mmol/l; female: 2.00 (±2 SD: 1.27–2.73) mmol/l.
[b] Historical control values: male: 1.96 (±5th percentile: 1.02–3.25) mmol/l; female: 1.52 (±5th percentile: 0.71–2.68) mmol/l.

high-dose F_1 males, mainly as a result of four males (Nos 353, 366, 367 and 372) with very low individual values for these organ weights, which also exhibited extremely low body weights.

At 70 ppm, no treatment-related histopathological changes were detected. A slight increase in the severity of the adrenal vacuolation was seen at 350 ppm and above in F_0 and F_1 females and at 1750 ppm in F_0 and F_1 males. In F_0 males, a higher incidence of foamy vacuolation of the jejunal villi epithelium was noted at the high dose. In F_1 males, a higher incidence of testicular and epididymal atrophy was found at the high dose, which was associated with epididymal oligospermia and partly atrophic prostate and seminal vesicles. Testicular and epididymal findings in the other groups were regarded as incidental and within the normal historical range. A slight increase in the severity of vacuolation or degeneration of luteal cells (a morphologically normal luteal regression) was noted in 1750 ppm F_1 females. Although the toxicological relevance was unclear, a relationship with treatment could not be excluded (Table 24).

Evaluation of reproductive performance and function showed no treatment-related effects on the fertility index, gestation index, total number of pups born, number of stillbirths, postimplantation loss or number of live litters produced in either generation, mean gestation interval, estrous cycle length, number of females with prolonged or abnormal cycles, day of vaginal patency in F_1 females, per cent motile sperm or per cent abnormal sperm in both generations, or testicular spermatid and epididymal sperm count in high-dose F_0 males. Decreases in the insemination (mating) index (of 8%), testicular spermatid count (of 33%) and epididymal sperm count (of 18%) were noted in high-dose F_1 males. Both the epididymal sperm and testicular spermatid counts were below the historical control ranges (provided in Amendment III to the study report). It was indicated by the study authors that the mean testicular and epididymal sperm counts were reduced due to the fact that four males in this dose group (Nos 353, 366, 367 and 372), which also had extremely low body weights, had no sperm. In addition, it was noted that there were five high-dose animals that had lower sperm or spermatid levels, which may have also contributed to the decreases in these sperm parameters at the high dose. No alteration in sperm count was found for F_1 males at the middle dose (Table 25).

Evaluation of offspring parameters showed no treatment-related or adverse effects on survival indices (live birth, viability and lactation), sex ratios, live litter size, clinical signs, relative organ weights (brain, thymus and spleen) or gross findings. During the 28-day lactation period, decreases in pup body weight were observed in mid- and high-dose pups of both generations. Decreases were noted throughout lactation in high-dose male F_1 (by 9–23%) and F_2 (by 6–17%) pups, and these pups also gained 24% and 17% less weight, respectively, than controls. Decreases in body weight and body weight gain were noted in high-dose F_1 (body weight: by 5–21%; body weight gain: by 22%)

Table 24. Selected microscopic findings in F_0 and F_1 parental animals

Organ/lesion	Incidence (no. of animals with lesion)[a,b]			
	Dietary concentration (ppm)			
	0	70	350	1750
F_0 generation – males				
Small intestine – epithelial vacuolation	4 (1.3)	0	5 (1.0)	17** (1.4)
Adrenal cortex – vacuolation	24 (2.0)[c]	24 (1.9)	23 (1.8)	25 (2.7)
F_0 generation – females				
Small intestine – epithelial vacuolation	6 (1.3)	7 (1.3)	5 (1.2)	10 (1.4)
Adrenal cortex – vacuolation	22 (1.2)	24 (1.2)	24 (1.6)	25 (2.1)
F_1 generation – males				
Small intestine – epithelial vacuolation	18 (1.3)	15 (1.5)	24 (1.7)	19 (1.6)
Adrenal cortex – vacuolation	22 (1.9)	25 (2.0)	25 (2.1)	24 (2.8)[c]
Testes – diffuse atrophy	0	1 (4.0)	1 (4.0)	4† (3.5)
Prostate – atrophy	0	0	0	3 (2.3)
Epididymides – oligospermia	0	1 (5.0)	1 (5.0)	4† (4.5)
Epididymides – atrophy	0	1 (4.0)	1 (4.0)	4† (3.8)
F_1 generation – females				
Small intestine – epithelial vacuolation	10 (1.5)	12 (1.4)	13 (1.2)	17* (1.6)
Ovaries – vacuolation/degeneration of luteal cells	21 (1.4)	24 (1.3)	25 (1.3)	23 (1.8)
Adrenal cortex – vacuolation	22 (1.2)	20 (1.2)	22 (1.5)	24 (2.1)

From Eiben (2000, 2002, 2003, 2004)

† $P < 0.055$; * $P < 0.05$; ** $P < 0.01$ (calculated by the reviewer)

[a] Number examined = 25 (except where otherwise noted).

[b] Average severity of lesions presented in parentheses, as reported by the study authors: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, 5 = massive.

[c] Number examined = 24.

Table 25. Spermatid and sperm counts in F_0 and F_1 males

	Dietary concentration (ppm)				Historical control data[a]
	0	70	350	1750	
F_0					
Mean number of spermatids/mg testis	48 760	—	—	46 217	Mean: 49 557 ± 2723 Range: 45 346–53 361
Mean number of sperm/mg epididymis	780 617	—	—	755 865	Mean: 10 268 523 ± 125 304 Range: 887 819–1 243 528
F_1					
Mean number of spermatids/mg testis	47 971	—	47 606	37 087 (77)[b]	Mean: 51 203 ± 601 Range: 50 841–51 897
Mean number of sperm/mg epididymis	889 967	—	982 994	733 837 (82)[b]	Mean: 1 077 723 ± 155 937 Range: 909 309–1 217 101

From Eiben (2000, 2002, 2003, 2004)

[a] Historical data from six studies conducted between 2000 and 2003; for the F_1 generation, there were data from only three of these studies.

[b] Per cent of control.

Table 26. Developmental landmarks in F_1 animals

Dietary concentration (ppm)	Mean age of sexual maturation[a] (days)	
	Preputial separation	Vaginal opening
0	35.4 ± 2.5	37.0 ± 2.7
70	35.0 ± 1.4	37.4 ± 3.4
350	35.4 ± 2.0	37.0 ± 2.9
1750	37.4 ± 1.8*	37.6 ± 2.1

From Eiben (2000, 2002, 2003, 2004)
* $P < 0.05$
[a] Mean ± standard deviation.

and F_2 (body weight: by 12–21%; body weight gain: by 22%) female pups during lactation. Mid-dose F_1 pups had decreased body weights (males: by 2–6%; females: by 3–9%) throughout lactation, whereas mid-dose F_2 pups had decreased body weights (males: by 5%; females: by 7%) only on the day of birth. Sexual maturation as measured by day of preputial separation was delayed by 2 days in high-dose F_1 males (Table 26). The Meeting noted that all observed treatment-related adverse effects on the reproductive system in this study were observed at the highest dose tested (1750 ppm) in second-generation (F_1) males and females, but not in first-generation (F_0) parental animals. This may suggest that early life stage exposure to spirodiclofen may have been a critical factor in eliciting this response, even though a number of the adverse outcomes were not observed until the animals were mature. However, this remains uncertain, considering that F_1 animals began consuming treated diet at an earlier age, experienced a longer duration of dosing and thus were exposed to a higher overall average dose of spirodiclofen compared with the F_0 generation.

The NOAEL for parental toxicity was 70 ppm, equal to 5.2 mg/kg bw per day for males and 5.5 mg/kg bw per day for females, based on the following findings at 350 ppm: decreased body weight in F_0 males, decreased body weight gain during premating in F_0 females and increased severity of adrenal cortical vacuolation in F_0 and F_1 females. The NOAEL for reproductive toxicity was 350 ppm, equal to 26.2 mg/kg bw per day for males and 27.6 mg/kg bw per day for females, based on delayed sexual maturation, decreased testicular spermatid and epididymal sperm counts (oligospermia), atrophy of the testes, epididymides, prostate and seminal vesicles in F_1 males and increased severity of ovarian luteal cell vacuolation/degeneration in F_1 females at 1750 ppm. The NOAEL for offspring toxicity was 70 ppm, equal to 5.2 mg/kg bw per day for males and 5.5 mg/kg bw per day for females, based on decreased body weight and body weight gain in F_1 male and female pups at 350 ppm (Eiben, 2000, 2002, 2003, 2004).

(b) Developmental toxicity

Rats

In a GLP-compliant developmental toxicity study, spirodiclofen (purity 97.9%) was administered to pregnant Wistar (Hsd Cpb:WU) rats (28 per dose) by gavage (in 0.5% aqueous carboxymethyl cellulose) at dose levels of 0, 100, 300 or 1000 mg/kg bw per day from days 6 through 19 of gestation. On gestation day (GD) 20, all surviving dams were sacrificed and examined grossly, and fetuses were delivered by caesarean section. The number of corpora lutea, implantations, early resorptions, late resorptions, and live and dead fetuses, as well as uterine and placental weight and appearance of the placenta, were assessed at caesarean section. Each fetus was weighed and examined externally for abnormalities, including the palate, and for sex determination. Approximately one half of the fetuses in each litter were examined viscerally by sectioning according to a modified Wilson technique. The remaining one half of the fetuses in each litter were eviscerated and processed for skeletal (bone and cartilage) examination.

No treatment-related deaths or clinical signs of toxicity were observed. There were no treatment-related effects on maternal body weight, body weight gains, food consumption or water intake. Maternal necropsy observations were unremarkable.

No treatment-related differences were noted between the treated and control groups for numbers of corpora lutea and implantations, placental and gravid uterine weights, live fetuses per dam, resorptions, fetal sex ratios, and preimplantation or postimplantation losses. Fetal body weights were similar between the treated and control groups. There were no treatment-related increases in any malformations or in the total number of fetuses or affected litters with malformations. A single case of microphthalmia was noted in the high dose group, which is considered a common spontaneous malformation in the Wistar rat and was thus considered incidental. A slight increase in the fetal and litter incidence of renal pelvis dilatation was observed at 1000 mg/kg bw per day. The study authors concluded that it was within the provided historical control range and therefore was not related to treatment. The Meeting requested additional information regarding the terminology used to describe the renal pelvis dilatation. The manufacturer indicated that "slight renal pelvis dilatation" refers to "renal papilla present though shorter giving the impression of enlarged renal pelvis", whereas "renal pelvis dilatation" (which the Meeting has referred to as "full" renal pelvis dilatation) was stated to refer to "lack of renal papilla, and/or reduced renal parenchyma with large renal pelvis". It had been noted that in the 2 years immediately preceding the study, there had been an apparent decrease in the background incidence of renal pelvis dilatation (Table 27). However, additional historical control data (from 1999 to 2001; Table 27) were also provided by the manufacturer, which showed that in the 2 years following the study with spirodiclofen, there was a rise in the background incidence of slight renal pelvis dilatation. Data were also provided for a range-finding study (Table 28) in which it was noted that although there was an increase in "full" renal pelvis dilatation on a fetal basis, there was no increase on a litter basis (five of six fetuses with this finding were from a single litter). Consequently, the study author concluded that there was no treatment-related effect on this finding in the range-finding study. A reference was provided (Woo & Hoar, 1972) that suggested that renal pelvis dilatation is a "transitory condition" representing "a normal aspect of renal development", although Klaus (2009) noted that an increased incidence of this finding "may be related to delayed fetal development". Klaus (2009) also noted that kidneys will generally be normal at weaning. The Meeting noted that the tendency for this finding to be transient was also supported more recently when a review of the background incidence of this finding indicated that renal pelvis dilatation is more often observed in fetuses than in pups, indicating that this finding should be considered a variation, rather than a malformation (Solecki et al., 2003). The Meeting noted, however, that histopathological examinations of these lesions were not conducted to confirm their nature and considered the increase at the high dose to be related to treatment. However, this finding was considered to be marginal, given that the finding occurred at a high dose (1000 mg/kg bw per day) and that the incidence was within the historical control range for studies conducted before and after the current study.

Variations in ossification rates of numerous bones were common findings in treated and control groups (Table 29); the general trend was towards slightly greater ossification in the treated groups compared with the controls, particularly in some cranial bones (e.g. interparietal, supraoccipital and temporal). Such findings were thought to be incidental and likely related to an incidentally lower body weight of the control fetuses. There appeared to be a dose-dependent increase in asymmetrical sternebrae (fourth) that reached statistical significance at the high dose; however, the fetal (high dose: 10.3%) and litter (high dose: 46.2%) incidence of asymmetrical fourth sternebrae from the current study were within the range of the historical control data provided for skeletal findings (fetal incidence: 5.3–12.5%; litter incidence: 19.2–53.8%) and were therefore not considered to be related to treatment by the study authors. However, the Meeting concluded that while marginal, the increase at the high dose could not be discounted as being related to treatment. The Meeting noted that due to the nature of this finding (Makris et al., 2009), it would be considered unlikely to be caused by a single dose.

Table 27. Incidence of renal pelvis dilatation in rats

Obervations	Dose (mg/kg bw per day)			
	0	100	300	1000
Fetuses				
Number of fetuses examined	133	137	129	144
Renal pelvis dilatation – slight (*N*)	8	5	4	14
Renal pelvis dilatation – full (*N*)	0	0	1	1
Renal pelvis dilatation – total (slight plus full) (*N*)	8 (6.0)[a]	5 (3.6)[a]	5 (3.9)[a]	15 (10.4)[a]
Historical control mean (1995–1996)[b] (%)	*6.0 (1.6–11.0)[a]*			
Historical control mean (1997)[c] (%)	*2.4 (2.2–2.8)[a]*			
Historical control mean (1999–2001)[d] (%)	*7.7 (2.1–11.0)[a]*			
Historical control mean from all studies (1995–2001)[e] (%)	*6.0 (2.1–11.0)[a]*			
Litters				
Number of litters examined	24	25	25	26
Renal pelvis dilatation – slight (*N*)	4	4	2	8
Renal pelvis dilatation – full (*N*)	0	0	1	1
Renal pelvis dilatation – total (slight plus full) (*N*)	4 (17)[f]	4 (16)[f]	3 (12)[f]	8 (31)[f]
Historical control mean (1995–1996)[b]	*41.4 (18.2–69.2)[f]*			
Historical control mean (1997)[c]	*21.3 (20.0–22.2)[f]*			
Historical control mean (1999–2001)[d]	*44.8 (14.0–77.0)[f]*			
Historical control mean from all studies (1995–2001)[e]	*38.6 (14.0–77.0)[f]*			

From Klaus (2000)

[a] Percentage of fetuses affected in parentheses.

[b] Mean incidence of unilateral/bilateral (slight) renal pelvis dilatation from seven studies in Wistar rats conducted between 1995 and 1996.

[c] Mean incidence of unilateral/bilateral (slight) renal pelvis dilatation from four studies in Wistar rats conducted in 1997. Although an additional four studies conducted in 1998 showed fetal and litter incidences similar to those from the studies conducted in 1997, the overall numbers of fetuses and litters examined were not provided, and therefore percentages of litters and fetuses affected could not be calculated.

[d] Mean incidence of unilateral/bilateral (slight) renal pelvis dilatation from eight studies in Wistar rats conducted between 1999 and 2001.

[e] Mean incidence of unilateral/bilateral (slight) renal pelvis dilatation from all provided studies in Wistar rats conducted between 1995 and 2001.

[f] Percentage of litters affected in parentheses.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day. The study authors considered the NOAEL for developmental toxicity to be 1000 mg/kg bw per day. However, the Meeting identified the NOAEL for developmental toxicity as 300 mg/kg bw per day, based on the marginal increases in renal pelvis dilatation and asymmetrical fourth sternebrae at 1000 mg/kg bw per day (Klaus, 2000).

Rabbits

In a GLP-compliant developmental toxicity study, spirodiclofen (purity 97.9–98.5%) was administered to pregnant Himalayan CHBB:HM rabbits (22 per dose) by gavage (in a 0.5% carboxymethyl cellulose sodium salt suspension) at dose levels of 0, 100, 300 or 1000 mg/kg bw per day from days 6 through 28 of gestation. On GD 29, all surviving does were sacrificed and examined grossly, and fetuses were delivered by caesarean section. The number of corpora lutea, implantations, early resorptions, late resorptions, and live and dead fetuses, as well as uterine and placental weight and appearance of the placenta, were assessed at caesarean section. Each fetus was weighed and examined for external abnormalities and for sex determination. Fetuses were examined viscerally by a modified Staples technique, including a transverse section through the brain in about 50% of the

Table 28. Incidence of renal pelvis dilatation from a rat range-finding study with spirodiclofen

Observations	Incidence (no. of fetuses/litters affected)			
	Dose (mg/kg bw per day)			
	0	100	300	1000
Number of fetuses/litters examined	22/6	21/4	34/6	31/6
Renal pelvis dilatation – slight	6/3	4/3	5/2	6/3
Renal pelvis dilatation – "full"	2/2	2/2	2/2	6/2
Renal pelvis dilatation – total (slight plus "full")	8/5	6/3	7/3	12/4

From Klaus (2000)

Table 29. Incidence of selected skeletal findings in rats

Observations	Incidence (no. of fetuses/litters with finding)			
	Dose (mg/kg bw per day)			
	0	100	300	1000
Number of fetuses/litters examined	144/24	154/25	143/25	156/26
Incomplete ossification				
- Parietal bone (bilateral)	10/5	8/4	0**/0	3/3
- Interparietal bone	31/14	16*/10	8**/4**	10**/7
- Supraoccipital bone	13/6	11/8	5/5	1**/1
- Temporal bone (bilateral)	8/5	4/2	1/1	0**/0
Asymmetrical fourth sternebrae[a,b]	3 (2) / 3 (12.5)	10 (6.5) / 10 (40)	12 (8.4) / 10 (40)	16* (10) / 12 (46)

From Klaus (2000)

* $P < 0.05$; ** $P < 0.01$

[a] Mean historical control incidence of asymmetrical fourth sternebrae on a fetal (litter) basis: 8.1% ± 2.3% (39.0% ± 11.5%) from nine studies conducted between 1996 and 1997.

[b] Per cent of total given in parentheses.

fetuses. The eviscerated carcasses were processed for skeletal examination, including cartilage staining. For approximately half of the fetuses, the head was examined via a transverse section through the brain and left intact for skeletal processing and examination; for the remainder of the fetuses, the heads were sectioned by a modified Wilson technique for an evaluation of internal cranial structures.

There were no treatment-related mortalities in maternal animals. Transient decreases in food consumption and faeces were observed in middle and high dose groups. In addition, light-coloured faeces were seen in 14 high-dose animals compared with none in the control or other treatment groups. Increased incidences of alopecia and discoloured urination, accompanied by a decrease in water consumption, were also noted in high-dose does. No statistical differences in absolute body weights were found between the treated and control groups at any time during the study. However, weight loss during GDs 6–9 was significantly greater in the middle (decreased by 55.8 g) and high dose groups (decreased by 72.7 g) compared with the control group (decreased by 23.7 g). Body weight loss and reduced faeces correlated with decreased food consumption values at the middle and high doses (which were 72% and 58%, respectively, of the control levels for GDs 6–9). Body weight changes and food consumption by the low dose group were similar to those of the control group throughout the study. One female in the high dose group aborted on GD 20 after showing distinct signs of systemic toxicity (cold ears, severe body weight loss and nearly no feed intake, as well as

distinct liver lobulation at necropsy). This one abortion resulted in a marginal decrease in gestation rate at the high dose (95.2% versus 100% in controls).

No statistically significant differences were noted between the treated and control groups for numbers of corpora lutea, implantations, live fetuses, resorptions, fetal sex ratios, placental weight and appearance, and preimplantation or postimplantation losses. Fetal body weights were similar between the treated and control groups. No treatment-related external, visceral or skeletal malformations or deviations were observed in fetuses. Although there was an increase in the number of fetuses with distinct liver lobulation in the high dose group (14 versus 3 in controls; 10.1% versus 2.2%; Table 30), there was no increase on a litter basis (three high-dose versus two control litters affected), and the incidence was reportedly within the historical control range. It was noted that the fetal incidence for liver lobulation at the high dose (10.1%) was above the historical control range provided in the study report (0–7.1%) from 18 studies conducted between 1991 and 1996; however, the litter incidence (15%) was within the historical control range if all pilot studies were included (as high as 33% if pilot studies with only three litters are included), but not if these pilot studies were excluded (range without the pilot studies is 0–6.7%). It appeared that the inclusion of the pilot studies artificially inflated the litter incidence of this finding, as only three litters were examined. The manufacturer provided additional historical control data from 1997 to 1998 (Klaus, 2009; added to Table 30). The range reported from these studies was 0–11.6% on a fetal basis and 0–19.0% on a litter basis from nine studies conducted during 1997 and 1998 (note: there were no incidences of this finding noted in the four studies conducted in 1998). When the additional historical control data from 1997 and 1998 are taken into consideration, the incidence of liver lobulation (on both a fetal and litter basis) is within the reported historical control range. Considering this and the fact that the incidence of liver lobulation is significantly increased only on a fetal basis (but not on a litter basis) in the study under review, the Meeting concurred with the study authors that this increase is not likely to be related to treatment with spirodiclofen.

Based on the study results, the NOAEL for maternal toxicity was 100 mg/kg bw per day, based on increased body weight loss and decreased food consumption at 300 mg/kg bw per day. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the limit dose (Holzum, 1998).

2.6 Special studies

(a) Neurotoxicity studies

Rats

In a GLP-compliant acute neurotoxicity study, a single dose of spirodiclofen (purity 97.7–97.9%; mixed batch) was administered to groups of fasted 9-week-old Wistar (Crl:WI(HAN)BR) rats (12 of each sex per group) by gavage in 0.5% methyl cellulose/0.4% Tween 80 in deionized water at doses of 0, 200, 500 or 2000 mg/kg bw. Rats were observed for 14 days, during which time clinical observations were assessed daily and body weights were recorded weekly. Neurobehavioural assessment, which included a FOB and motor/locomotor activity testing in a figure-eight maze, was performed 1 week prior to treatment, approximately 4 h after administration of the dose and 7 and 14 days following treatment. At study termination, all animals were subjected to gross necropsy, and six animals of each sex per group were euthanized and perfused (in situ) for neuropathological examination; brain weights were also recorded for these animals. Of the perfused animals, only the control and high dose groups were subjected to histological evaluation of brain and peripheral nervous system tissues. 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 effects that were considered treatment related.

Based on the absence of effects in this study, the NOAEL for spirodiclofen in rats is the limit dose of 2000 mg/kg bw (Sheets & Gilmore, 2000).

Table 30. Incidence of liver lobulation in fetal rabbits

Observation	Dose (mg/kg bw per day)			
	0	100	300	1000
Fetuses				
Number of fetuses examined	138	137	136	139
Distinct liver lobulation (*N*)	3 (2.2)[a]	0	1	14* (10.1)[a]
Historical control mean (range)[b] (%)	*0.5 ± 1.7 (0–7.1)*			
Historical control mean (range)[c] (%)	*2.2 ± 4.3 (0–11.6)*			
Historical control mean (range)[d] (%)	*1.1 ± 2.7 (0–11.6)*			
Litters				
Number of litters examined	21	22	20	20
Distinct liver lobulation (*N*)	2 (9.5)[e]	0	1	3 (15)[e]
Historical control mean (range)[b] (%)	*0.6 ± 1.9 (0–6.7)*			
Historical control mean (range) including pilot studies[f] (%)	*2.0 ± 7.0 (0–33.3)*			
Historical control mean (range)[c] (%)	*4.1 ± 8.0 (0–19)*			
Historical control mean (range)[d] (%)	*2.6 ± 6.6 (0–19)*			

From Holzum (1998)

* $P \leq 0.05$

[a] Percentage of fetuses affected in parentheses.

[b] Mean (± standard deviation) historical control incidence of liver lobulation from 18 studies conducted between 1991 and 1996.

[c] Mean (± standard deviation) historical control incidence of liver lobulation from nine studies conducted between 1997 and 1998 (Klaus, 2009).

[d] Mean (± standard deviation) historical control incidence of liver lobulation from all main studies (27 in total) conducted between 1991 and 1998.

[e] Percentage of litters affected in parentheses.

[f] Mean historical control incidence of liver lobulation from 18 main studies conducted between 1991 and 1996, as well as 5 pilot studies conducted in 1996, which used only three litters. Fetal incidence could not be calculated from the pilot studies, as the total number of fetuses examined was not provided.

Note: Positive control data, which were referenced but not included in the study report, were provided by the manufacturer in response to a request by the JMPR (Sheets, 1993).

In a GLP-compliant subchronic neurotoxicity study, spirodiclofen (purity 97.4–97.8%; mixed batch) was administered to groups of young adult Wistar rats (12 of each sex per dose) in the diet (in 1% corn oil) at doses of 0, 100, 1000 or 12 500 ppm (equal to 0, 7.2, 70.3 and 1088.8 mg/kg bw per day for males and 0, 9.1, 87.3 and 1306.5 mg/kg bw per day for females) for 13 weeks. Detailed clinical observations and body weight recordings were conducted weekly, and food consumption was determined every 1–2 days. Neurobehavioural assessments, which included FOB and motor activity testing (figure-eight maze), were performed in all 12 animals of each sex per group. Ophthalmological examinations were conducted on all animals at pretest and prior to terminal sacrifice. At study termination, all animals were subjected to gross necropsy and perfused, with six of each sex per dose subjected to histopathological evaluation of brain, peripheral nervous system tissues, eyes (with optic nerves) and skeletal muscle. Brain weights were also recorded for the animals selected for histopathological assessment.

There was no mortality at any dose level prior to scheduled terminal sacrifice. At 12 500 ppm, urine staining was observed in both sexes. High-dose females also showed oral staining and red-tinged stains at locations such as paws, snout, forelimbs and ears. Body weights were reduced for males (by 24%) and females (by 15%) at the high dose, but not at lower doses. Food consumption was reduced at the high dose for both sexes. For the FOB, there were compound-related effects in both sexes at the

high dose, but not at any lower doses. Observations associated with treatment were limited to various stains (including oral, urine, nasal, on paws and/or on ears) in both sexes, and the high-dose animals of both sexes tended to have slightly lower landing food splay and grip strength measurements on some test occasions. These small differences in grip strength and landing foot splay were attributed to the large decrease in body weight in these animals. No compound-related effects on motor and locomotor activity in the figure-eight maze were noted at any dose level for males. High-dose females showed a consistent slight decrease in motor and locomotor activity during week 4. Habituation was not affected by treatment. No changes were noted in ophthalmic findings. Compound-related gross lesions at terminal sacrifice were limited to increased incidence of ventral staining at the highest dose level in both sexes. Absolute brain weights were not affected at any dietary level. Relative brain weights were increased, which was likely due to the observed decrease in body weight at this dose level. No compound-related microscopic lesions were noted in either sex at the highest dose.

The NOAEL was 1000 ppm (equal to 70.3 mg/kg bw per day in males and 87.3 mg/kg bw per day in females) based on decreased body weights, decreased food consumption and increased urine staining in both sexes and decreased motor and locomotor activity for females (during week 4) at 12 500 ppm (Sheets & Gilmore, 2001).

Note: Positive control data, which were referenced but not included in the study report, were provided by the manufacturer in response to a request by the JMPR (Sheets, 1993; Sheets & Armintrout, 2000).

In a GLP-compliant developmental neurotoxicity study, spirodiclofen (purity 96.8–97.1%; mixed batch) was administered to pregnant Wistar Hannover rats (30 per dose) continuously from GD 0 to lactation day (LD) 21 in the diet at nominal doses of 0, 70, 350 or 1500 ppm (equal to 0/0, 6.5/14.0, 32.1/69.7 and 135.9/273.8 mg/kg bw per day [gestation/lactation]). On postnatal day (PND) 4, litters were standardized to four pups of each sex per litter; excess pups were killed and discarded. Pups were weaned on PND 21, after which time all animals received untreated diet. F_1 pups were assigned to subgroups (1 pup per litter per group [approximately 16 pups of each sex per dose]) in order to evaluate developmental landmarks (preputial separation or vaginal patency), brain weights, learning and memory (passive avoidance after weaning and an M-water maze task on PND 60), motor activity (in a figure-eight maze) and acoustic startle habituation. Serum cholesterol level was measured in the dams (LD 21) and offspring (PNDs 4 and 21), and neural tissues were collected from 10 rats of each sex per dietary level (representing approximately 20 litters) on PND 21 (brain only) and at study termination (approximately 75 days of age) for microscopic examination and morphometry.

No treatment-related effects were observed in the dams for mortality, clinical signs, FOB, serum cholesterol levels, reproductive performance or postmortem examinations. No treatment-related differences were noted in body weights, body weight gains or food consumption during the gestation period. During lactation, a statistically significant body weight decrease (of 5%) was observed in the 1500 ppm dams on LD 21. Food consumption was decreased (by 8%) in the 1500 ppm dams during LDs 7–14. However, body weight changes within each group did not show significant differences compared with the control group.

Treatment had no adverse effects on offspring survival, clinical signs or developmental landmarks. During preweaning, body weights were decreased in the 1500 ppm pups on PNDs 17 and 21 (by 5–8%). Body weight gains were decreased at 1500 ppm in both sexes at most intervals (by 6–18%), and overall (PNDs 0–21) body weight gain was decreased in each sex at 1500 ppm (by 9%). During post-weaning, body weights recovered in the 1500 ppm group. Post-weaning food consumption was similar between the treated and control males. No treatment-related effects were observed on FOB, motor activity or auditory startle reflex assessments. No treatment-related differences in the passive avoidance tests or the water maze task (Table 31) were reported at any dose by the study authors. For postmortem examination, no significant differences in absolute brain weight or

Table 31. Water M-maze performance for F_1 rats in the original developmental neurotoxicity study[a]

Session/parameter		Dietary concentration (ppm)			
		0	70	350	1500
Males					
Session 1 – Learning	No. of animals	16	16	16	16
	Trials to criterion	7.0 ± 2.1	7.2 ± 2.8	7.8 ± 2.9	6.9 ± 1.4
	Trial 1 – Errors	1.0 ± 1.0	0.6 ± 0.8	1.1 ± 1.1	0.8 ± 0.9
	Trial 1 – Duration (s)	19.4 ± 16.9	16.6 ± 14.0	21.6 ± 13.9	20.3 ± 14.2
	Trial 2 – Errors	0.3 ± 0.4	0.5 ± 0.7	0.6 ± 0.8 (2-fold increase)[b]	0.8 ± 1.0 (2.7-fold increase)[b]
	Trial 2 – Duration (s)	12.9 ± 7.0	12.2 ± 11.5	15.7 ± 15.1 (+18)[c]	17.3 ± 14.6 (+34)[c]
	Failed to meet criterion	1	0	0	0
Session 2 – Memory	No. of animals	15	16	16	16
	Trials to criterion	6.5 ± 2.8	5.6 ± 1.3	5.4 ± 0.7	5.3 ± 0.6
	Trial 1 – Errors	0.5 ± 0.7	0.4 ± 0.9	0.3 ± 0.7	0.3 ± 0.6
	Trial 1 – Duration (s)	9.7 ± 4.9	6.1 ± 4.0	8.9 ± 6.8	8.5 ± 5.5
	Trial 2 – Errors	0.3 ± 0.7	0.1 ± 0.3	0.3 ± 0.8	0.1 ± 0.3
	Trial 2 – Duration (s)	6.1 ± 3.8	3.5 ± 0.9	4.7 ± 3.8	4.9 ± 3.0
	Failed to meet criterion	0	0	0	0
Females					
Session 1 – Learning	No. of animals	16	16	16	16
	Trials to criterion	6.8 ± 2.5	7.1 ± 2.2	7.7 ± 2.6 (+13)[c]	7.6 ± 2.9 (+12)[c]
	Trial 1 – Errors	0.8 ± 1.3	0.8 ± 0.9	1.3 ± 1.0 (+62)[c]	1.1 ± 1.6 (+38)[c]
	Trial 1 – Duration (s)	15.3 ± 10.4	17.2 ± 13.9 (+12)[c]	22.6 ± 11.7 (+48)[c]	19.4 ± 14.0 (+27)[c]
	Trial 2 – Errors	0.3 ± 0.6	0.6 ± 0.6	0.4 ± 0.9	0.6 ± 0.6
	Trial 2 – Duration (s)	8.1 ± 3.0	10.5 ± 6.6 (+30)[c]	11.4 ± 8.8 (+41)[c]	12.1 ± 6.2 (+49)[c]
	Failed to meet criterion	0	0	0	1
Session 2 – Memory	No. of animals	16	16	16	15
	Trials to criterion	5.9 ± 1.9	8.5 ± 3.7* (+47)[c]	7.3 ± 2.3* (+26)[c]	8.9 ± 4.5 (53%)
	Trial 1 – Errors	0.1 ± 0.3	0.8 ± 1.1	0.3 ± 0.6	0.3 ± 0.5
	Trial 1 – Duration (s)	8.2 ± 6.1	12.3 ± 10.3	9.4 ± 7.8	7.5 ± 4.1
	Trial 2 – Errors	0	0.1 ± 0.5	0.1 ± 0.5	0.2 ± 0.6
	Trial 2 – Duration (s)	5.3 ± 1.9	6.6 ± 7.7	6.8 ± 4.7	5.2 ± 4.5
	Failed to meet criterion	0	0	0	4

From Sheets & Lake (2006)

* $P < 0.05$

[a] The water M-maze was repeated in a second, modified, developmental neurotoxicity study; results are presented in Table 33 below. Results are expressed as mean ± standard deviation.

[b] Value presented in parentheses represents increase compared with controls.

[c] Per cent change compared with controls given in parentheses.

Table 32. Selected microscopic brain measurements in F_1 rats (n = 10)[a]

Parameter	Brain measurement (mm)		
	Dietary concentration (ppm)		Historical control range[b]
	0	1500	
Males – PND 21			
Caudate putamen	3.05 ± 0.032	3.04 ± 0.118	2.96–3.30
Parietal cortex	1.88 ± 0.094	1.85 ± 0.077	1.81–2.03
Hippocampal gyrus	1.50 ± 0.070	1.46 ± 0.091	1.44–1.77
Males – Termination			
Caudate putamen	3.42 ± 0.182	3.29 ± 0.288 (−4)[c]	3.12–3.51
Parietal cortex	1.88 ± 0.088	1.75 ± 0.148* (−6)[c]	1.74–1.93
Hippocampal gyrus	1.62 ± 0.155	1.67 ± 0.148	1.57–1.74
Females – PND 21			
Caudate putamen	3.08 ± 0.078	3.00 ± 0.076* (−3)[c]	2.88–3.27
Parietal cortex	1.90 ± 0.138	1.89 ± 0.105	1.82–2.06
Hippocampal gyrus	1.45 ± 0.184	1.55 ± 0.114 (+7)[c]	1.38–1.69
Females – Termination			
Caudate putamen	3.38 ± 0.114	3.47 ± 0.063* (+3)[c]	3.21–3.55
Parietal cortex	1.82 ± 0.044	1.81 ± 0.061	1.76–1.88
Hippocampal gyrus	1.53 ± 0.176	1.62 ± 0.145 (+6)[c]	1.48–1.76

From Sheets & Lake (2006)
* $P < 0.05$
[a] Mean ± standard deviation.
[b] No details were provided regarding the source of the reference range data.
[c] Per cent change compared with controls given in parentheses.

cerebrum and cerebellum lengths were observed at any dose in either sex at any time point. There were no changes noted in morphometric measurements for day 21 male pups (Table 32). For day 21 females, there was a statistically significant decrease in the caudate putamen measurement. In day 70 pups, there was a statistically significant increase in the caudate putamen measurement in females and a decrease in the parietal cortex measurement in males. The morphometric changes were considered by the study authors to be unrelated to treatment, as the microscopy and gross measurements revealed no morphological changes in these areas, the changes were noted in one sex only and the changes were not noted in both time periods examined.

The study authors considered the NOAEL for maternal toxicity to be 350 ppm (equal to 32.1 mg/kg bw per day), based on decreased body weight, body weight gain and food consumption during lactation at 1500 ppm. However, the Meeting noted that although the body weight decrease in maternal animals may be treatment related, it was not considered biologically significant (decrease of 5%). Therefore, the Meeting concluded that the maternal NOAEL was 1500 ppm (equal to 135.9 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 350 ppm (equal to 32.1 mg/kg bw per day), based on decreased body weight during lactation at 1500 ppm (Sheets & Lake, 2006).

In a GLP-compliant modified developmental neurotoxicity study, spirodiclofen (purity 98.3–97.5%; mixed match) was administered to groups of pregnant Wistar Hannover rats (30 per dose) continuously from GD 0 to LD 21 in the diet at nominal doses of 0, 70, 350 or 1500 ppm (equal to 0, 5.4, 28.6 and 119.2 mg/kg bw per day during gestation and 0, 13.0, 65.7 and 262.1 mg/kg bw per day

during lactation). The United States Environmental Protection Agency and Health Canada (Pest Management Regulatory Agency) had reviewed the original developmental neurotoxicity study (Sheets & Lake, 2004; summarized above), requested that brain measurements be taken from low- and mid-dose animals and raised questions about the M-maze results for treated female offspring tested on PND 60. As the brains from the low- and mid-dose animals had been stored for an extended period in fixative and were therefore subject to shrinkage relative to tissues for control and high-dose animals, these tissues were considered by the manufacturer to be unsuitable for comparison. Consequently, the manufacturer chose to conduct a modified developmental neurotoxicity study. The purpose of this present study was to provide data needed to address the aforementioned issues without unnecessarily repeating tests that had been performed in the original developmental neurotoxicity study. On PND 4, litters with a minimum of seven pups, including at least three per sex, were culled to yield, as closely as possible, four males and four females. Subsets of surviving offspring, representing at least 18–20 litters per dietary level, were subjected to evaluation using the following observations and measurements: detailed clinical observations, body weight, tests of spatial learning and memory (i.e. M- and Cincinnati water mazes) beginning on PND 60 (±2 or 4 days, respectively) and an ophthalmic examination. The M-maze test was repeated under the same conditions as in the original study, and a separate group of animals was tested using the Cincinnati water maze, a test of spatial learning and memory with a more complex configuration. Whole-brain tissue was collected from 10 of each sex per dietary level (representing 20 litters) on PND 21 and at study termination (approximately 75 days of age) for morphometry.

No mortalities or significant treatment-related effects were observed in maternal animals during the study. Slight decreases in food consumption (of 4–5%) and body weight gain (of 4%) were noted during lactation; however, these findings were not considered to be adverse owing to the slight magnitude of the effects and lack of statistical significance. In addition, no treatment-related effects on reproductive performance were observed.

Offspring viability, litter size and sex ratio were not affected by exposure to the test substance. No treatment-related mortalities or adverse clinical signs of toxicity were observed in the offspring during lactation or post-weaning, nor were there any treatment-related ophthalmological findings. Decreased mean pup body weights in high-dose males and females were observed from PNDs 17 to 21 and were statistically significant at PND 21 only (decreased by 8%). Overall body weight gain was significantly decreased in high-dose males and females from PNDs 4 to 21 (by 9–10%). At post-weaning, mean body weight was decreased in high-dose males from PNDs 29 to 50 (by 5–6%), with statistical significance reached on PNDs 35 and 42. No significant body weight changes were observed during post-weaning (PNDs 28–70) for females.

In the M-maze (Table 33), control and treated animals of both sexes demonstrated acquisition during the learning phase. One control female, one low-dose male, one mid-dose female and one high-dose female failed to meet the criterion during the learning phase. In addition, one high-dose male was inadvertently not tested for acquisition. Animals were not included for testing in the retention phase if acquisition was not demonstrated. There were no compound-related effects on acquisition or retention in males or females at any dietary level. Furthermore, there were no statistical differences from control at any dietary level on either test occasion. In the Cincinnati water maze (Table 34), control and treatment group animals of both sexes demonstrated acquisition and retention. No significant treatment-related findings were observed in the Cincinnati water maze. Postmortem examination did not reveal any significant differences in absolute brain weight in males or females on PND 21 or at study termination. Relative brain weights were increased (by 9%) in high-dose males at study termination, which was considered to be due to the decrease in terminal body weight observed in this dose group, rather than a direct effect on the brain. There were no statistically significant changes in brain morphometric measurements (Table 35).

Table 33. Water M-maze performance for F_1 rats in the modified developmental neurotoxicity study[a]

Session/parameter		Dietary concentration (ppm)			
		0	70	350	1500
Males					
Session 1 – Learning	No. of animals	16	16	16	15
	Trials to criterion	6.4 ± 1.4	6.3 ± 2.5	7.9 ± 2.4 (+23)[b]	7.6 ± 2.1 (+19)[b]
	Trial 1 – Errors	0.6 ± 0.6	0.6 ± 0.9	0.6 ± 0.8	1.2 ± 1.1 (2-fold increase)[c]
	Trial 1 – Duration (s)	9.9 ± 4.2	13.6 ± 6.4 (+13)[b]	14.8 ± 7.8 (+50)[b]	15.9 ± 9.5 (+60)[b]
	Trial 2 – Errors	0.4 ± 0.5	0.3 ± 0.4	0.7 ± 0.8 (+75)[b]	1.0 ± 1.0 (2.5-fold increase)[c]
	Trial 2 – Duration (s)	10.4 ± 6.1	9.5 ± 5.6	16.2 ± 14.2 (+56)[b]	14.9 ± 9.9 (+43)[b]
	Failed to meet criterion	0	1	0	0
Session 2 – Memory	No. of animals	16	15	16	15
	Trials to criterion	5.4 ± 1.0	6.5 ± 2.4	5.6 ± 1.4	6.7 ± 2.6 (+24)[b]
	Trial 1 – Errors	0.2 ± 0.4	0.3 ± 0.5	0.3 ± 0.7	0.1 ± 0.3
	Trial 1 – Duration (s)	7.3 ± 4.0	8.6 ± 6.7	9.5 ± 7.3	6.2 ± 4.3
	Trial 2 – Errors	0	0	0	0
	Trial 2 – Duration (s)	5.1 ± 2.8	4.2 ± 2.2	4.5 ± 2.6	4.3 ± 1.6
	Failed to meet criterion	0	0	0	0
Females					
Session 1 – Learning	No. of animals	16	16	15	16
	Trials to criterion	7.6 ± 2.6	7.3 ± 2.1	8.1 ± 3.1	7.3 ± 2.9
	Trial 1 – Errors	0.8 ± 1.0	0.8 ± 1.1	0.9 ± 1.2	0.4 ± 0.8
	Trial 1 – Duration (s)	15.3 ± 10.4	12.3 ± 7.3	13.2 ± 8.1	12.6 ± 6.5
	Trial 2 – Errors	0.7 ± 1.1	0.9 ± 1.1	0.7 ± 1.3	0.5 ± 1.0
	Trial 2 – Duration (s)	12.7 ± 10.1	12.6 ± 7.0	10.7 ± 8.7	10.2 ± 6.5
	Failed to meet criterion	1	0	1	1
Session 2 – Memory	No. of animals	15	16	14	15
	Trials to criterion[c]	7.9 ± 3.6	8.4 ± 3.9	8.0 ± 3.8	8.9 ± 4.2 (+13)[b]
	Trial 1 – Errors	0.0 ± 0.0	0.4 ± 0.9	0.4 ± 0.6	0.1 ± 0.4
	Trial 1 – Duration (s)	4.9 ± 2.9	7.7 ± 9.4 (+57)[b]	9.1 ± 7.4 (+86)[b]	6.9 ± 4.9 (+41)[b]
	Trial 2 – Errors	0.2 ± 0.6	0.3 ± 0.6	0.1 ± 0.5	0.1 ± 0.4
	Trial 2 – Duration (s)	6.1 ± 7.3	5.7 ± 3.3	6.9 ± 9.3	4.9 ± 4.8
	Failed to meet criterion	0	1 (6.3)	2 (14)	3 (20)

From Gilmore, Sheets & Hoss (2007)

[a] Mean ± standard deviation.

[b] Per cent change compared with controls given in parentheses.

[c] Compared with controls.

[d] For females that failed to meet the criterion within 15 trials, a value of 15 was assigned for the purpose of calculating the mean number of trials for the group.

Table 34. Cincinnati water maze performance for F_1 rats[a]

Session		Dietary concentration (ppm)							
		0		70		350		1500	
		Latency	Errors	Latency	Errors	Latency	Errors	Latency	Errors
Males									
Learning phase	Day 1	300 ± 0	18.5 ± 3.1	286 ± 35	17.3 ± 3.8	300 ± 0	17.8 ± 4.2	289 ± 28	16.6 ± 3.9
	Day 2	241 ± 84	17.6 ± 7.8	227 ± 94	15.8 ± 5.8	217 ± 85	16.0 ± 6.4	269 ± 31	16.7 ± 3.9
	Day 3	175 ± 117	9.9 ± 7.6	118 ± 109	6.0 ± 5.1	104 ± 60	6.5 ± 4.9	171 ± 100	9.7 ± 5.7
	Day 4	80 ± 60	4.8 ± 5.5	100 ± 105	4.8 ± 7.2	48 ± 19	1.8 ± 1.8	96 ± 96	4.1 ± 5.6
	Day 5	52 ± 37	2.0 ± 3.3	63 ± 86	2.1 ± 4.2	48 ± 37	0.9 ± 1.5	44 ± 25	1.1 ± 1.1
	Overall[b]	169.6	10.6	158.8	9.2	143.4	8.6	173.8	9.6
Retention phase	Day 12	42 ± 19	1.0 ± 1.1	40 ± 26	1.9 ± 3.3	40 ± 16	0.4 ± 0.5	35 ± 21	0.5 ± 0.9
Females									
Learning phase	Day 1	278 ± 68	20.2 ± 6.4	280 ± 34	22.3 ± 4.0	296 ± 14	21.7 ± 5.9	282 ± 57	21.5 ± 5.8
	Day 2	242 ± 82	17.9 ± 6.0	218 ± 83	16.7 ± 6.3	224 ± 85	17.5 ± 8.2	178 ± 102	16.1 ± 9.4
	Day 3	154 ± 99	11.2 ± 7.6	112 ± 73	8.0 ± 7.0	110 ± 108	5.8 ± 6.1	85 ± 59	6.1 ± 5.3
	Day 4	81 ± 66	4.5 ± 5.5	42 ± 18	2.0 ± 1.4	66 ± 86	2.8 ± 4.7	52 ± 45	2.3 ± 2.8
	Day 5	41 ± 30	1.4 ± 1.9	24 ± 63	0.5 ± 0.7	57 ± 87	1.4 ± 2.0	35 ± 19	1.7 ± 1.2
	Overall[b]	159.2	11.0	135.2	9.9	150.6	9.8	126.4	9.5
Retention phase	Day 12	72 ± 69	4.9 ± 7.4	57 ± 50	1.8 ± 2.9	49 ± 46	2.2 ± 3.6	89 ± 85	5.0 ± 5.8

From Gilmore, Sheets & Hoss (2007)
[a] Mean ± standard deviation.
[b] Calculated by the reviewer.

Table 35. Selected microscopic brain measurements in F_1 rats from the modified developmental neurotoxicity study

Parameter	Brain measurement (mm)[a]				
	Dose (ppm)				Historical control[b]
	0	70	350	1500	
Males – PND 21 (*n* = 10)					
Caudate putamen	3.03 ± 0.141	3.04 ± 0.100	3.00 ± 0.100	2.93 ± 0.141 (−3)[c]	2.79–3.30
Cerebellum	5.16 ± 0.300	4.97 ± 0.374	5.02 ± 0.200	4.91 ± 0.283 (−5)[c]	4.03–4.90
Hippocampal gyrus	1.78 ± 0.100	1.71 ± 0.100	1.71 ± 0.200	1.76 ± 0.141	1.44–1.75
Parietal cortex	1.97 ± 0.100	2.00 ± 0.100	1.98 ± 0.100	1.90 ± 0.100	1.82–2.03
Frontal cortex	1.86 ± 0.100	1.92 ± 0.055	1.89 ± 0.141	1.78 ± 0.100	1.66–1.93
Males – PND 75 (*n* = 10)					
Caudate putamen	3.29 ± 0.100	3.28 ± 0.173	3.19 ± 0.173	3.32 ± 0.100	3.12–3.51
Cerebellum	5.05 ± 0.265	4.77 ± 0.374	4.78 ± 0.424	5.10 ± 0.173	3.83–4.96
Hippocampal gyrus	1.72 ± 0.100	1.76 ± 0.100	1.74 ± 0.100	1.67 ± 0.100 (−3)[c]	1.55–1.81
Parietal cortex	1.93 ± 0.055	1.96 ± 0.063	1.94 ± 0.045	1.97 ± 0.055	1.74–1.93
Frontal cortex	1.78 ± 0.100	1.79 ± 0.100	1.74 ± 0.100	1.82 ± 0.063	1.58–1.91

Table 35 (contd)

Parameter	Brain measurement (mm)[a]				
	Dose (ppm)				Historical control[b]
	0	70	350	1500	
Females – PND 21 (*n* = 10)					
Caudate putamen	2.93 ± 0.100	2.99 ± 0.200	2.96 ± 0.100	3.00 ± 0.100	2.83–3.23
Cerebellum	4.97 ± 0.346	4.76 ± 0.346	4.86 ± 0.283	4.90 ± 0.224	3.99–5.10
Hippocampal gyrus	1.69 ± 0.100	1.66 ± 0.141	1.64 ± 0.173	1.59 ± 0.100 (−6)[c]	1.38–1.74
Parietal cortex	1.96 ± 0.100	1.97 ± 0.100	1.96 ± 0.100	1.98 ± 0.055	1.70–2.02
Frontal cortex	1.84 ± 0.141	1.89 ± 0.063	1.87 ± 0.100	1.85 ± 0.063	1.64–1.96
Females – PND 75 (*n* = 10)					
Caudate putamen	3.22 ± 0.100	3.14 ± 0.200	3.17 ± 0.141	3.18 ± 0.100	3.21–3.55
Cerebellum	4.84 ± 0.200	4.85 ± 0.173	4.77 ± 0.245	4.64 ± 0.200 (−4)[c]	3.98–4.98
Hippocampal gyrus	1.75 ± 0.141	1.70 ± 0.100	1.71 ± 0.141	1.69 ± 0.173 (−3)[c]	1.48–1.76
Parietal cortex	1.84 ± 0.032	1.84 ± 0.100	1.88 ± 0.045	1.82 ± 0.045	1.68–1.88
Frontal cortex	1.75 ± 0.045	1.70 ± 0.100	1.73 ± 0.032	1.67 ± 0.100 (−5)[c]	1.65–1.84

From Gilmore, Sheets & Hoss (2007)

[a] Mean ± standard deviation.

[b] Historical control data were provided for 17 (terminal pup data) and 19 (PND 21 pup data) studies conducted between 2000 and 2006.

[c] Per cent change compared with controls given in parentheses.

Based on the results from the study, the NOAEL for maternal toxicity was 1500 ppm (equal to 119.2 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 350 ppm (equal to 28.6 mg/kg bw per day), based on decreases in preweaning body weight and body weight gain in both sexes and reduced post-weaning body weight in males at 1500 ppm (Gilmore, Sheets & Hoss, 2007).

Positive control data for validation of the developmental neurotoxicity studies (which were referenced, but not included, in the study report) were provided by the manufacturer (Sheets, 2001; Sheets & Lake, 2001). The data pertaining to the M-maze are summarized in Table 36. The Meeting noted that there was considerable variability in the M-maze data from both the validation studies as well as the spirodiclofen studies under review, which makes it difficult to clearly identify treatment-related changes in this parameter in any of these studies. Positive control data were also provided for the Cincinnati water maze (Table 37). However, data were provided only for males, and, as such, the study was not validated for females (Sheets, 2007).

The Meeting noted that small morphometric changes in the following brain regions were observed in the first study (Sheets & Lake, 2006) at the high dose (low and middle doses were not examined): caudate putamen, hippocampal gyrus and parietal cortex. In the second study (Gilmore, Sheets & Hoss, 2007), morphometry was performed at all dose levels, and small changes were noted at the high dose in the caudate putamen, cerebellum and hippocampal gyrus. Although some of the same brain regions appeared to be affected in both studies, the observed changes were small (3–7%), did not attain statistical significance in many cases and were not consistent between studies, and thus they were not considered to be related to treatment (see Table 35).

In tests of learning and memory, the Meeting noted that females required an increased number of trials at all dose levels to meet the criterion during the memory phase of the original M-maze. In addition, four high-dose females failed to complete the retention task (five consecutive errorless trials

Table 36. M-maze performance[a] of rats from validation studies with methimazole[b] and scolopamine[c]

Session/parameter		Methimazole concentration (mg/ml)		Scopolamine concentration (mg/kg bw)	
		0	0.1	0	1.0
Males					
Session 1 – Learning	No. of animals	23	20	12	12
	Trials to criterion	7.3 ± 2.5	9.5 ± 3.3* (+30)[d]	7.8 ± 3.2	9.5 ± 4.1 (+22)[d]
	Trial 1 – Errors	0.9 ± 0.9	0.9 ± 0.7	0.9 ± 1.1	0.9 ± 0.5
	Trial 1 – Duration (s)	18.4 ± 11.9	24.3 ± 17.6 (+32)[d]	23.0 ± 17.7	28.4 ± 15.5 (+23)[d]
	Trial 2 – Errors	0.5 ± 0.8	0.8 ± 0.9 (+38)[d]	0.6 ± 0.9	0.6 ± 0.7
	Trial 2 – Duration (s)	15.0 ± 13.1	20.3 ± 12.0* (+35)[d]	20.3 ± 13.2	20.2 ± 14.0
	Failed to meet criterion	1 (4)[e]	2 (10)[e]	1 (8)[e]	3 (25)[e]
Session 2 – Memory	No. of animals	22	18	10	9
	Trials to criterion	5.8 ± 1.9	6.5 ± 2.3 (+12)[d]	6.8 ± 2.3	8.2 ± 2.8 (+21)[d]
	Trial 1 – Errors	0.2 ± 0.4	0.8 ± 1.2 (4-fold increase)[f]	0.2 ± 0.4	0.3 ± 0.5
	Trial 1 – Duration (s)	9.6 ± 11.7	17.3 ± 18.2 (+80)[d]	11.8 ± 6.1	15.1 ± 6.8 (+28)[d]
	Trial 2 – Errors	0 ± 0	0.2 ± 0.4	0.3 ± 0.7	0.3 ± 0.5
	Trial 2 – Duration (s)	4.2 ± 1.7	7.8 ± 6.7 (+86)[d]	8.9 ± 8.0	13.4 ± 8.7 (+50)[d]
	Failed to meet criterion	0	0	0	0
Females					
Session 1 – Learning	No. of animals	23	21	12	12
	Trials to criterion	8.1 ± 2.8	10.0 ± 3.0 (+23)[d]	10.1 ± 3.5	11.9 ± 3.0 (+18)[d]
	Trial 1 – Errors	0.7 ± 0.7	0.7 ± 0.7	1.2 ± 1.7	0.5 ± 0.5
	Trial 1 – Duration (s)	16.1 ± 9.5	17.6 ± 11.9 (+9)[d]	24.0 ± 14.6	18.4 ± 6.2*
	Trial 2 – Errors	0.6 ± 1.1	1.0 ± 1.5 (+67)[d]	0.7 ± 0.9	0.6 ± 0.7
	Trial 2 – Duration (s)	14.0 ± 13.1	19.7 ± 15.9 (+41)[d]	18.6 ± 14.6	19.1 ± 10.7
	Failed to meet criterion	0	2	2	5
Session 2 – Memory	No. of animals	23	19	10	6
	Trials to criterion	8.2 ± 3.7	9.5 ± 4.4 (+16)[d]	9.4 ± 3.9	11.2 ± 2.9 (+19)[d]
	Trial 1 – Errors	0.6 ± 1.0	0.5 ± 0.9	0.4 ± 0.7	0.5 ± 0.8
	Trial 1 – Duration (s)	10.3 ± 9.7	12.1 ± 10.3 (+17)[d]	14.0 ± 11.3	30.3 ± 18.5 (1.2-fold increase)[f]
	Trial 2 – Errors	0.1 ± 0.5	0.3 ± 0.6	0.3 ± 0.7	0.7 ± 0.8
	Trial 2 – Duration (s)	6.6 ± 5.0	8.1 ± 5.8 (+23)[d]	11.0 ± 9.2	14.0 ± 10.0 (+30)[d]
	Failed to meet criterion	3	3	0	0

From Sheets (2001); Sheets & Lake (2001)

* $P < 0.05$

[a] Mean ± standard deviation.

[b] Methimazole was administered to pregnant Wistar rats via drinking-water on GD 16 to LD 10; F_1 rats performed M-maze at beginning at PND 60. Doses of methimazole administered to maternal rats were calculated based on test substance intake and body weight data provided in the study report: days 0–7 (GD 16–22): 14.2 mg/kg bw per day; days 7–14 (LD 0–7): 18.9 mg/kg bw per day; days 14–21 (LD 10): 20.8 mg/kg bw per day (Sheets & Lake, 2001).

[c] Scopolamine was administered (subcutaneously) to 8-week-old Wistar rats 30–60 min prior to testing (Sheets, 2001).

[d] Per cent change compared with controls given in parentheses.

[e] Per cent of total given in parentheses.

[f] Compared with controls.

Table 37. Cincinnati water maze performance with scopolamine in males (n = 12)[a]

Session		Dose (mg/kg bw)							
		Control		0.3		0.6		1.2	
		Latency	Errors	Latency	Errors	Latency	Errors	Latency	Errors
Learning phase	Day 1	282 ± 42	20.3 ± 4.6	300 ± 0	40.5 ± 8.1	293 ± 24	38.6 ± 10.4	300.0 ± 0	45.2 ± 5.5
	Day 2	206 ± 83	14.0 ± 5.7	263 ± 69	30.0 ± 13.5	274 ± 48	37.4 ± 12.1	284 ± 55	45.1 ± 15.0
	Day 3	110 ± 82	6.4 ± 4.3	165 ± 100	15.3 ± 11.4	209 ± 117	22.9 ± 15.7	218 ± 99	29.2 ± 19.8
	Day 4	69 ± 48	3.0 ± 2.9	98 ± 84	6.0 ± 5.9	143 ± 107	14.2 ± 12.4	216 ± 98	24.6 ± 16.9
	Day 5	40 ± 23	1.2 ± 1.8	104 ± 106	6.8 ± 8.9	158 ± 96	14.8 ± 13.9	117 ± 118	20.8 ± 18.8
	Overall	141	9.0	186	19.7*	216*	25.6*	239*	33.0*
Retention phase	Day 12	76 ± 46	2.6 ± 3.0	129 ± 69	9.6 ± 6.3	139 ± 90	10.5 ± 7.8*	179 ± 82*	15.0 ± 9.7*

From Sheets (2007)
* $P < 0.05$
[a] Scopolamine was administered (by intraperitoneal injection) to Wistar rats 30 min prior to acquisition testing. Results are mean ± standard deviation.

during the memory phase) in this test (Sheets & Lake, 2006; see Table 31). In the second study (Gilmore, Sheets & Hoss, 2007), there was a slight increase in the number of trials to meet the criterion for high-dose females during the memory phase in the M-maze task, and there were a few females in the treated groups that failed to meet the criterion of five consecutive errorless trials in the retention phase (incidence of 0, 1, 2 and 3; see Table 33). It should be noted that in groups in which animals failed to complete the retention task, the values for mean trials to criterion are underestimated, as an arbitrary value of 15 trials was assigned for those animals that failed to complete the task within 15 trials. A Cincinnati water maze was also conducted in the second study. While there were no treatment-related findings noted in this maze, interpretation of these results was limited due to the novelty of this test to the conducting laboratory, the fact that the test was not validated for females and the considerable variability noted in the data in the current study for females, which led to uncertainty regarding the sensitivity of the test to detect treatment-related changes (see Table 34).

Overall, the Meeting concluded that the results of these studies did not indicate any treatment-related findings on neurotoxicity parameters in offspring, as the findings were inconsistent, and the considerable variability in the data limited their interpretation.

(b) Supplemental studies on mechanisms of endocrine effects

Several in vitro and short-term toxicity studies were conducted with spirodiclofen and its enol metabolites in order to further investigate the endocrine effects observed in the toxicology studies, which effects included vacuolation of steroid tissues (adrenal gland and Leydig cells) in mice, rats and dogs, signs of androgen deprivation in dogs (e.g. immature prostate and germinal epithelium, as well as oligospermia and aspermia), Leydig cell hypertrophy or hyperplasia in mice, rats and dogs, and neoplastic lesions in steroid-sensitive tissues, the testes and uterus, in the 2-year rat study (see sections 2.2(a), 2.3 and 2.5(a) above).

In vitro studies to investigate androgen and/or estrogen receptor-mediated mechanisms

In a non-GLP in vitro study, the effects of spirodiclofen and the enol (BAJ 2510) and 4-hydroxy-enol (at concentrations of 10^{-11}–10^{-5} mol/l) metabolites were investigated in a series of reporter gene and receptor binding assays. In the reporter gene assays, human androgenic (human prostate-derived PALM cells) or estrogenic (human breast cancer–derived MVLN cells) cell lines transfected with a

reporter gene (luciferase) coupled with a hormone response element were used in the presence or absence of estrogen (MVLN cells) or testosterone (PALM cells). The affinity of spirodiclofen and the metabolites for the estrogen receptor was tested in competitive receptor binding assays with human alpha and beta estrogen receptors with a fluorescence-labelled estrogen. In all test systems, estradiol or methyltestosterone was used as a positive control.

Spirodiclofen and the enol metabolite were not cytotoxic to either of the cell lines used, whereas the 4-hydroxy-enol metabolite showed slight cytotoxicity to MVLN cells. Spirodiclofen and its metabolites did not show any androgenic or antiandrogenic activity in PALM cells. In MVLN cells, spirodiclofen and the 4-hydroxy-enol did not show any estrogenic or antiestrogenic activity, whereas the enol metabolite showed some contradictory results. The effects of the enol metabolite appeared to be dependent on pH, such that estrogenic and antiestrogenic effects were noted at pH 6–6.5. In the receptor binding assays, none of the test compounds bound to either of the receptor subtypes under normal pH conditions (pH 7.5); however, at a lower pH (pH 6–6.5), the enol metabolite had a slight binding activity. The study author concluded that spirodiclofen and the enol and 4-hydroxy-enol metabolites did not display estrogenic, antiestrogenic, androgenic or antiandrogenic activity under conditions of physiological pH (Schmuck, 1999).

In vitro studies to investigate effects on cholesterol esterase

In a non-GLP in vitro radiometric assay, the possible inhibition of cholesterol esterase, an enzyme required for intestinal absorption and liberation of cholesterol from intracellular lipid pools, by spirodiclofen and the enol metabolite, BAJ 2510, was investigated. Based on findings in steroid-sensitive tissues noted in the in vivo studies, it was considered likely that steroidogenesis would be affected by treatment with spirodiclofen. One of the earliest steps in the synthesis of steroid hormones is the release of the precursor cholesterol from cholesterol ester stores by cholesterol esterase. The purpose of the current in vitro study was to investigate whether spirodiclofen or BAJ 2510 could inhibit steroid hormone synthesis at the level of this enzyme, by investigating its potential to inhibit the cholesterol esterase–catalysed release of radiolabelled fatty acid from a radiolabelled cholesterol ester. The potential for spirodiclofen to act as an alternative substrate for cholesterol esterase was also examined. Spirodiclofen (0.1–100 µmol/l) or BAJ 2510 (0.1–1000 µmol/l) was incubated with commercially available porcine pancreatic cholesterol esterase in the presence of radiolabelled cholesterol oleate for the cholesterol esterase inhibition experiments. Spirodiclofen (5 or 50 µmol/l) was also incubated in the absence of radiolabelled cholesterol oleate for the alternative substrate experiments.

A dose-dependent inhibition of cholesterol esterase was noted with spirodiclofen in two separate experiments (median inhibitory concentrations [IC_{50}s] of 12 and 43 µmol/l). BAJ 2510 inhibited the enzyme only at 1000 µmol/l. Spirodiclofen was not found to act as a substrate for cholesterol esterase, and thus inhibition of the enzyme by acting as a competing substrate could not be substantiated as the inhibitory mode of action. Results from in vivo studies demonstrated that spirodiclofen could not be detected in the plasma of spirodiclofen-treated animals (Wetzig & Ruehl-Fehlert, 2001). As BAJ 2510, the main plasma metabolite, displayed weak inhibitory potential on cholesterase esterase, interference with the synthesis of steroid hormones via effects on this enzyme was not supported by the results of this in vitro study (Freyberger, 2000).

In vitro studies to investigate effects on testosterone formation

In a non-GLP study, the effects of spirodiclofen and its enol metabolites (BAJ 2510, 3-hydroxy-enol and 4-hydroxy-enol) on testosterone secretion by rat testicular tissue maintained in dynamic organ culture were studied. Spirodiclofen (1.5–100 µmol/l) and the enol metabolites (1–1000 µmol/l) were incubated with rat testicular tissue in the presence of human chorionic gonadotrophin (hCG), dibutyryl-cyclic adenosine monophosphate (db-cAMP) or 25-hydroxycholesterol (25-OH-

cholesterol) and hCG, in order to investigate potential sites of action in the steroidogenic pathway of these test substances. 25-OH-cholesterol can enter the mitochondria without the need of active transport and thus can be used to circumvent potential inhibitory effects on cholesterol esterase and cholesterol transport into the mitochondria. Testosterone levels in the incubation medium were measured by radioimmunoassay.

Spirodiclofen suppressed hCG-stimulated testosterone formation in one of four repeat experiments and also suppressed cAMP-stimulated testosterone products (by 50% at 50 µmol/l). BAJ 2510 (≥10 µmol/l with hCG or db-cAMP; ≥100 µmol/l with hCG and 25-OH-cholesterol) strongly inhibited testosterone production under all conditions tested, whereas the hydroxy-enols produced moderate inhibition of hCG- and cAMP-stimulated testosterone at high doses only (1000 µmol/l). The fact that BAJ 2510 suppressed testosterone production even in the presence of 25-OH-cholesterol suggests that the site of inhibition is downstream of cholesterol transport into the mitochondria (Freyberger, 2001a).

In vitro studies to investigate effects on microsomal enzymes involved in testosterone synthesis

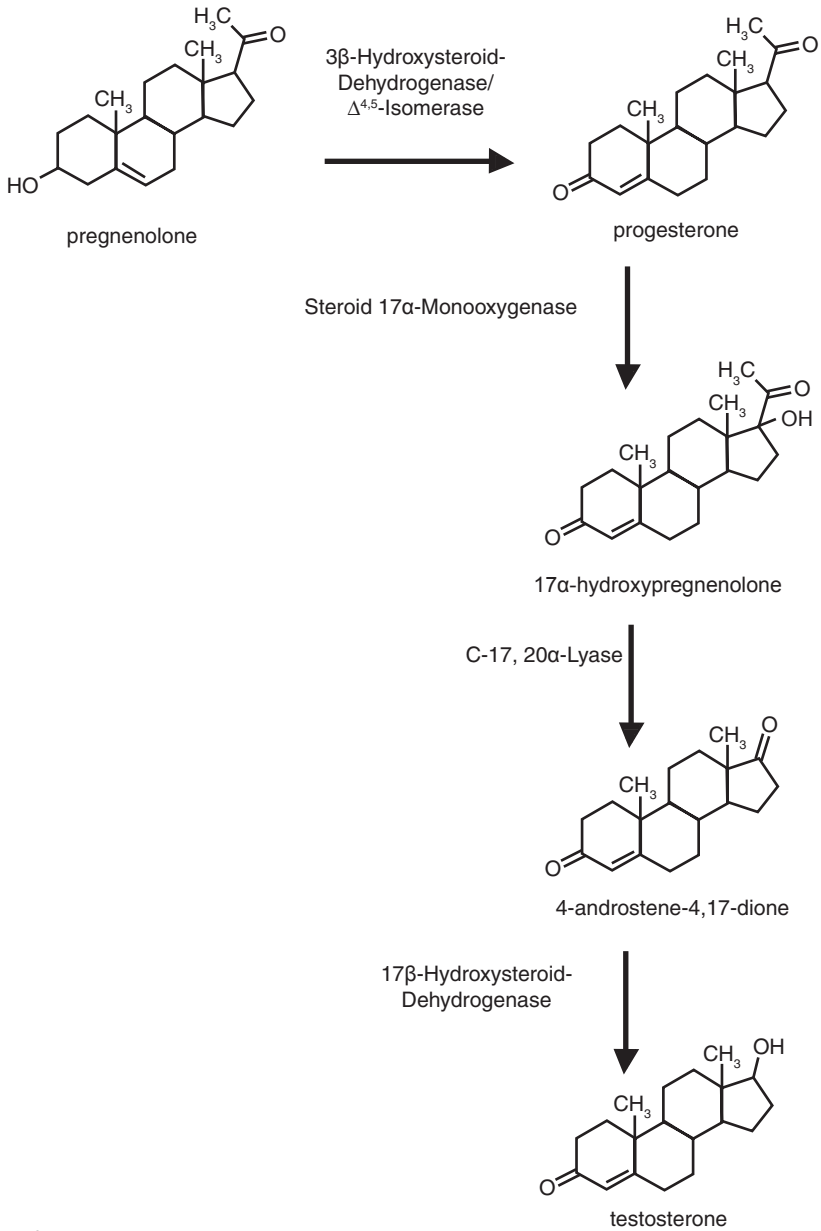
In order to investigate whether treatment with spirodiclofen interfered with microsomal cytochrome P450–dependent mono-oxygenases involved in steroid hormone synthesis, the effects of spirodiclofen and its metabolites BAJ 2510 (enol), 3-hydroxy-enol and 4-hydroxy-enol on steroid 17α-mono-oxygenase (involved in the conversion of progesterone to 17α-progesterone) and C-17,20-lyase (conversion of 17α-progesterone to androstenedione; Figure 2) were studied in a non-GLP in vitro study. Rat testicular microsomes served as an enzyme source. Microsomes were incubated with precursor steroids (progesterone or 17α-hydroxyprogesterone) in the presence of test compounds, and resultant products (17α-hydroxyprogesterone or androstenedione) were quantified by ultraviolet (UV) spectroscopy following HPLC separation from educts.

No inhibition of steroid 17α-mono-oxygenase occurred in the presence of spirodiclofen at 50 µmol/l (maximum non-precipitating concentration) or BAJ 2510 at 300 µmol/l. Slight inhibition of C-17,20-lyase was observed in the presence of spirodiclofen at 50 µmol/l and BAJ 2510 at 300 µmol/l, which produced 13% and 8% inhibition, respectively. Based on these results, it was concluded that inhibitory effects on these cytochrome P450–dependent microsomal mono-oxygenases in animals treated with spirodiclofen were unlikely to occur, as spirodiclofen is not detectable in plasma; although BAJ 2510 is present in micromole per litre concentrations, it did not significantly inhibit these enzymes in vitro. Further, in the in vitro experiments with rat testicular fragments, BAJ 2510 significantly decreased testosterone formation at concentrations as low as 10 µmol/l (Freyberger, 2001b).

In order to investigate whether treatment with spirodiclofen interfered with microsomal dehydrogenases involved in steroid hormone biosynthesis, the effects of spirodiclofen and its metabolites BAJ 2510 (enol), 3-hydroxy-enol and 4-hydroxy-enol on 3β-hydroxysteroid dehydrogenase-$\Delta^{4,5}$-isomerase (conversion of pregnenolone to progesterone) and 17β-hydroxysteroid dehydrogenase (conversion of androstenedione to testosterone; Figure 2) were studied in a non-GLP in vitro study. Rat testicular microsomes served as an enzyme source. Microsomes were incubated with precursor steroids (pregnenolone or androstenedione) in the presence of test compounds, and resultant products (progesterone or testosterone) were quantified by UV spectroscopy or liquid scintillation counting following HPLC separation from educts.

Spirodiclofen at 25 or 50 µmol/l inhibited the formation of progesterone by 20% and 22%, respectively, whereas BAJ 2510 (300 µmol/l), 3-hydroxy-enol (100 µmol/l) and 4-hydroxy-enol (100 µmol/l) failed to inhibit the formation of progesterone, demonstrating that spirodiclofen (at maximum non-precipitating concentrations), but not the enol metabolites, inhibited 3β-hydroxysteroid dehydrogenase-$\Delta^{4,5}$-isomerase by 20% in vitro. Spirodiclofen (50 µmol/l), BAJ 2510 (100 µmol/l), 3-hydroxy-enol (1000 µmol/l) and 4-hydroxy-enol (1000 µmol/l) failed to inhibit the transformation

Figure 2. Microsomal reactions of the testosterone-synthesizing pathway



From Freyberger (2001b)

of androstenedione to testosterone, indicating that none of the test compounds affect the 17β-hydroxysteroid dehydrogenase enzyme. Whereas spirodiclofen produced a moderate inhibition of 3β-hydroxysteroid dehydrogenase-$\Delta^{4,5}$-isomerase under the conditions of this study, the study author concluded that as plasma levels of the parent compound are not detectable in animals treated with spirodiclofen (Wetzig & Ruehl-Fehlert, 2001), it was unlikely that inhibition of this enzyme would occur in vivo (Freyberger, 2001c).

In vitro studies to investigate effects on mitochondrial enzymes involved in steroidogenesis

In a series of non-GLP in vitro assays, the effects of spirodiclofen and its metabolites, BAJ 2510 (enol), 3-hydroxy-enol and 4-hydroxy-enol, on the side-chain cleavage enzyme (cytochrome P450scc) were studied in freshly isolated rat testicular mitochondria. Pregnenolone formed by

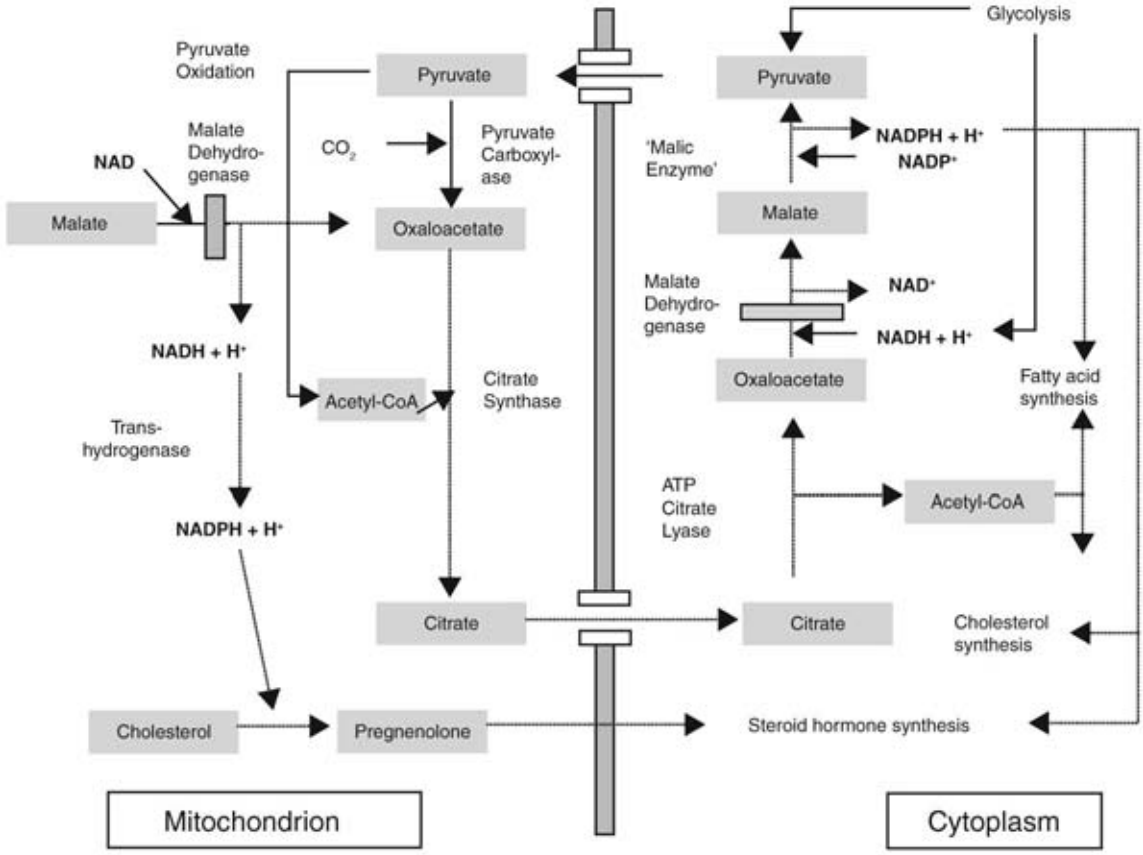
side-chain cleavage and released from mitochondria was enzymatically converted into progesterone and determined by HPLC. Effects on enzymes (malic enzyme [ME] and mitochondrial malate dehydrogenase [MD]) involved in the generation of reducing equivalents (reduced nicotinamide adenine dinucleotide phosphate [NADPH]) for the side-chain cleavage enzyme were studied as well using commercially available purified enzymes. Interactions with side-chain cleavage were also assessed in rat testicular tissue maintained in culture through measurements of progesterone and testosterone levels in the incubation medium. Finally, effects of BAJ 2510 on the availability of cytoplasmic reducing equivalents (produced through cytoplasmic MD) were studied. Cytoplasmic MD is part of the pyruvate/citrate shuttle that exports citrate from the mitochondria to the cytoplasm. There, citrate is degraded to acetyl coenzyme A (CoA) and oxaloacetate, which undergoes further reactions involving cytoplasmic MD, resulting in the formation of NADPH.

In the presence of 25-OH-cholesterol and malate, incubation of freshly isolated rat testicular mitochondria with BAJ 2510 at 100 µmol/l resulted in decreased side-chain cleavage (as evidenced by decreased progesterone formation). This inhibition could be lessened by increasing malate concentrations in the incubation medium and was almost reversed when malate was replaced by citrate. No interaction with the side-chain cleavage was observed in the presence of spirodiclofen at 50 µmol/l, 3-hydroxy-enol at 100 µmol/l or 4-hydroxy-enol at 100 µmol/l. These investigations suggested that BAJ 2510 affects steroidogenesis at the level of malate, which is involved in producing NADPH. As NADPH can be produced from malate through reactions involving either ME or mitochondrial MD, further assays were conducted to investigate which of these two enzymes might be affected by BAJ 2510. In these assays, BAJ 2510 inhibited mitochondrial MD, but not ME, as shown by a dose-dependent decrease in reduced nicotinamide adenine dinucleotide (NADH) oxidation by porcine heart mitochondrial MD in the presence of BAJ 2510. In cultured rat testicular tissue, BAJ 2510 decreased the hCG-stimulated synthesis of progesterone (50% inhibition with BAJ 2510 at 300 µmol/l), which was used as a measure of side-chain cleavage. Testosterone was also decreased by BAJ 2510, by a greater extent than was progesterone (90% inhibition with BAJ 2510 at ≥100 µmol/l). This suggests that BAJ 2510 may act at another site downstream of progesterone formation in the steroidogenic pathway. Similar findings were observed for ketoconazole, which is known to interfere with mitochondrial and microsomal target enzymes. Finally, in assays investigating potential effects on cytoplasmic MD, BAJ 2510 was found to inhibit this enzyme as well, as evidenced by a dose-dependent decrease in NADH oxidation by porcine heart cytoplasmic MD in the presence of BAJ 2510. This finding suggests that BAJ 2510 reduces the NADPH supply in the cytosol, resulting in strong inhibition of further NADPH-dependent reactions (Freyberger, 2001d).

The effect of BAJ 2510 on levels of reducing equivalents (NADH and NADPH) in rat testicular mitochondrial preparations was investigated in a series of non-GLP in vitro assays. As part of this study, a methodology to measure intramitochondrial NADH and NADPH was developed. Heat inactivation in the presence of detergent following addition of inhibitors of the respiratory chain/oxidative phosphorylation was used to suppress the decomposition of NADH and NADPH by mitochondrial lysates. NADH and NADPH levels in incubation medium were determined by reversed-phase ion pair chromatography and fluorescence detection.

In incubations with BAJ 2510 (300 µmol/l), statistically significant decreases in NADH concentrations were observed in the presence of varying concentrations of malate (decreases were 26–47%, 44–47% and 13–19% in the presence of malate at concentrations of 0.05 mmol/l, 0.5 mmol/l and 5 mmol/l, respectively). NADPH levels were also decreased by BAJ 2510, but less so, with increasing concentrations of malate (decreases were 26–41%, 25–31% and 2–6% in the presence of malate at concentrations of 0.05 mmol/l, 0.5 mmol/l and 5 mmol/l, respectively). Tartronate (5 mmol/l), a known inhibitor of mitochondrial ME, decreased NADH and NADPH levels to a similar extent (by approximately 25%), regardless of the concentration of malate used.

Figure 3. Proposed mode of action of the enol metabolite involving steroid inhibition[a]



Adapted from Kaune, Temerowski & Krautstrunk (2008)

[a] Consequences of mitochondrial MD inhibition are indicated by dotted lines; consequences of combined inhibition of mitochondrial and cytoplasmic MDs are indicated by dashed lines.

The study author concluded that these findings were consistent with the purported mechanism of action described for BAJ 2510, involving inhibition of intramitochondrial MD (Figure 3). It is thought that inhibition of this enzyme would result in decreased intramitochondrial NADH levels. Subsequently, less intramitochondrial NADPH would be formed from NADH (by transhydrogenation), and, finally, side-chain cleavage would be inhibited owing to a shortage of the relevant cofactor NADPH. As previously reported for the inhibition of side-chain cleavage by BAJ 2510, effects on NADPH and NADH levels also depended on malate concentrations, inhibition being most effective in the presence of low malate concentrations. The NADH levels tended to be more strongly inhibited by BAJ 2510 compared with the NADPH levels, which is in contrast to the findings with tartronic acid and again points to MD as the target of BAJ 2510. These findings further support the hypothesis of a competitive, reversible inhibition of MD by BAJ 2510 (Freyberger, 2002, 2003a).

In a second non-GLP study investigating the effect of BAJ 2510 on levels of reducing equivalents in the testes, varying concentrations of BAJ 2510 (purity 99.9%; 100, 200 or 300 µmol/l) were incubated with rat testicular mitochondrial preparations as described above (Freyberger, 2002) in the presence of malate (0.5 mmol/l). Tartronic acid was included as a positive control.

BAJ 2510 concentration-dependently decreased the overall amount of reducing equivalents (NADH + NADPH, statistically significant at all BAJ 2510 concentrations tested), NADH levels (statistically significant at BAJ 2510 concentrations of 200 and 300 µmol/l) and NADPH levels (statistically significant at all BAJ 2510 concentrations tested). In the presence of tartronic acid at

5 mmol/l, the overall amount of NADH + NADPH and NADPH concentrations were statistically significantly reduced by 30% and 34%, respectively, and NADH levels were non-significantly decreased by 28% (Freyberger, 2003b).

Short-term studies in rats

In a GLP-compliant subchronic toxicity study, spirodiclofen (purity 97.8%) was administered to groups of female Wistar rats (15 per dose) in the diet (containing 1% peanut oil) at dose levels of 0, 2500 or 10 000 ppm (equal to 0, 242.4 and 1209.6 mg/kg bw per day). Animals were dosed for 19 weeks and then monitored for a further 11 weeks of recovery with no test chemical. This study was conducted to investigate possible effects on circulating levels of hormones in female rats, in light of the increased incidence of uterine adenocarcinoma observed in the 2-year rat study (see section 2.3). Animals were inspected daily for mortality, and general clinical examinations, body weight measurements and feed intake determinations were conducted weekly. Blood samples were collected at weeks 7, 9, 11, 13, 17 and 19 during test substance administration and then at weeks 2 and 6 during the recovery period for determination of testosterone, estradiol, luteinizing hormone (LH) and progesterone. Vaginal smears were collected periodically throughout the study period for determination of estrous stage in order to attempt to collect blood samples when animals were in diestrus. All animals were subjected to gross pathological examination, and the liver, uterus, ovaries and adrenals were weighed. These tissues were fixed, but not subjected to histopathological examination.

Clinical signs noted during the dosing period were restricted to evidence of light discoloured faeces in some low-dose (7/15) and all high-dose females. Clinical signs resolved during the 11-week recovery period. Decreased body weight gain was noted for low-dose (by 7%) and high-dose (by 24%) animals during the treatment period. Food consumption was increased in treated animals in a dose-dependent manner (by 10% at the low dose and by 37% at the high dose). High-dose animals had significantly reduced testosterone, estradiol and progesterone levels during the 19 weeks of treatment (Table 38). It should be noted that testosterone levels in many of the samples were below the limit of detection (0.025 ng/ml). The estradiol levels were depressed to a greater extent than the progesterone levels, resulting in an increased estradiol to progesterone ratio in high-dose animals during the treatment period. There were no statistically significant differences in hormone levels during the recovery period; however, estradiol levels remained depressed (by 13–15%) following 6 weeks of recovery from treatment. Liver weights (absolute and relative to body weight) were reduced in high-dose animals, but the reductions were not regarded as biologically significant, whereas adrenal and ovary weights (absolute and relative) were increased in high-dose animals. Four high-dose females had grossly enlarged ovaries; however, microscopic examinations were not conducted (Andrews, 2001).

Table 38. Circulating levels of hormones in female rats during treatment and recovery

	Testosterone (ng/ml)	Estradiol (pg/ml)	LH (ng/ml)	Progesterone (ng/ml)	Estradiol/ progesterone
Treatment					
Week 7					
Control	0.02	92.20	1.19	8.36	11.02
2500 ppm	0.01	76.47	1.54	8.17	9.36
10 000 ppm	0.01	61.05***	0.84	5.47	11.16
Week 9					
Control	0.02	95.50	1.85	17.86	5.35
2500 ppm	0.01	84.73	1.59	10.36	8.19

Table 38 (contd)

	Testosterone (ng/ml)	Estradiol (pg/ml)	LH (ng/ml)	Progesterone (ng/ml)	Estradiol/ progesterone
10 000 ppm	0.01	74.59*	1.75	4.45*	16.81
Week 11					
Control	0.02	97.21	1.46	9.78	9.94
2500 ppm	0.02	93.67	1.74	5.41	17.31
10 000 ppm	0.02	83.85	1.15	4.17	20.11
Week 13					
Control	0.02	130.88	1.13	18.67	7.52
2500 ppm	0.01	137.38	1.15	9.95	13.80
10 000 ppm	0.01	110.61	1.14	4.99*	25.97*
Week 17					
Control	0.04	95.37	1.98	25.17	3.79
2500 ppm	0.02	95.34	1.76	13.30	7.78
10 000 ppm	0.02	91.83	1.82	5.61**	16.31*
Week 19					
Control	0.02	109.72	1.18	8.03	13.67
2500 ppm	0.01	93.46	1.00	9.67	9.93
10 000 ppm	0.01	71.37***	0.87	3.62	19.73
Recovery					
Week 2					
Control	0.01	93.25	1.95	10.65	8.75
2500 ppm	0.01	91.83	2.24	14.11	6.51
10 000 ppm	0.01	79.46	2.18	15.33	5.18
Week 6					
Control	0.01	82.71	2.31	7.22	11.45
2500 ppm	0.01	84.30	1.88	7.10	11.87
10 000 ppm	0.01	71.92	2.57	9.42	7.29

From Andrews (2001)
* P <0.05; ** P < 0.001; *** P < 0.0001

Short-term studies in dogs

In a short-term toxicity study, spirodiclofen (purity 97.9–98.6%) was administered to groups of male Beagle dogs (five per dose) in the diet at dose levels of 0, 100 or 2000 ppm (equal to 0, 2.9 and 55.9 mg/kg bw per day) for 8 weeks. This study was intended to clarify testicular effects previously observed in Beagle dogs exposed to spirodiclofen for 14 weeks. Clinical observations were conducted twice daily, food and water intakes were assessed daily and body weights were recorded weekly. Reflexes were assessed and body temperature and pulse were recorded before the start of the study and at week 8. Blood was collected for haematological and clinical chemistry investigations at pretest and during week 8. Additionally, blood samples were collected on a weekly basis for measurement of cholesterol, triglycerides, testosterone and LH levels. Blood samples from week 8 were also analysed for levels of α-tocopherol and ubiquinone. At study termination, all animals were subjected to gross necropsy, and the following organs were weighed and examined histopathologically: brain, heart, liver, lungs, spleen, adrenals, kidneys, pancreas, thyroid, pituitary, testes, prostate gland and thymus.

Table 39. Mean LH and testosterone levels in the dog

Dietary concentration (ppm)	Hormone levels (ng/ml)								
	Week								
	1	2	3	4	5	6	7	8	All
LH									
0	3.94	2.60	2.06	2.82	3.74	5.60	9.42	4.82	4.37
100	4.14	2.68	3.12	4.42	5.52	7.14	4.30	5.18	4.56
2000	9.76	9.48	6.80	5.66	7.08	9.62	22.86	9.78	10.13
Testosterone									
0	0.60	0.63	0.35	0.52	0.87	0.50	0.93	1.37	0.72
100	0.74	0.79	0.87	0.84	0.79	1.01	1.01	0.82	0.85
2000	0.54	0.61	0.22	0.52	0.49	0.94	0.71	0.73	0.59

From Wetzig (2001); Wetzig & Hartmann (2001a)

The activities of the following enzymes were determined in liver tissue: cytochrome P450, cytochrome P450–dependent mono-oxygenases (ECOD, EROD, ALD), EH and the conjugation enzymes (GST, GLUT). Testosterone metabolism (hydroxylation) was also examined in liver tissue of control and high-dose animals by measuring 6α-, 6β-, 16α-, 2α- and 2β-hydroxytestosterone levels, as well as androstenedione levels. This study was generally conducted in compliance with GLP; however, the testosterone hydroxylase assay and hormone determinations were not conducted according to GLP requirements. Levels of tocopherol, ubiquinone and dolichol were also reportedly determined in blood samples; however, the results from these investigations were not included in the study report.

There were no compound-related effects on mortality, clinical signs, reflexes, body temperature, body weight, food consumption or haematological parameters examined. Clinical chemistry investigations revealed increased levels of ASAT, ALAT, AP and GLDH at 2000 ppm compared with the controls. Cholesterol levels were reduced at 2000 ppm, but triglyceride levels were not affected by the treatment. There were no treatment-related effects on serum testosterone levels. However, serum LH concentrations were significantly increased and at least doubled at several weeks during the treatment period in the 2000 ppm animals, providing some evidence that the hypothalamic–pituitary–gonadal axis was affected by treatment with spirodiclofen (Table 39). The measurement of microsomal enzyme activities in the liver tissue revealed an induction of phase I enzymes, especially ECOD and ALD, in the 2000 ppm dose group. The testosterone hydroxylation assay revealed a slight induction of 16α-hydroxylation in the 2000 ppm group, which the study authors noted correlates to cytochrome P450 enzyme subtype CYP2Bl and is also comparable to the induction of ALD. All other in vitro metabolic steps of testosterone remained unchanged.

There were no gross pathological findings attributable to treatment with spirodiclofen. Increases in absolute and relative liver and adrenal weights were observed in the 100 and 2000 ppm groups. Prostate weights were slightly decreased in both treatment groups (Table 40). The histopathological examination revealed treatment-related findings in the liver, adrenal glands and reproductive organs (Table 41). Hepatocellular cytoplasmic change was seen in three males dosed at 2000 ppm. This finding is considered to be the morphological correlate of an induction of hepatocellular enzyme systems, reflecting an adaptive response to the metabolism of the test compound. Furthermore, minimal to slight hepatocellular single-cell necroses, reflecting a mild toxic injury to the liver cells, were observed in three out of five males of the high dose group. In the testes of all high-dose animals, hypertrophy and vacuolation of the Leydig cells were found.

Table 40. Selected absolute and relative organ weights in the dog

Dietary concentration (ppm)	Absolute (g) or relative (to body) weight (g/kg)				
	Liver	Adrenals	Prostate	Thyroid	Pancreas
Absolute weight					
0	402.8	1.15	4.78	0.92	30.6
100	461.6	1.59	4.04	1.11	34.2
2000	499.8	1.72	4.10	1.09	35.8
Relative weight					
0	33.2	0.0955	0.380	0.0760	2.51
100	37.3	0.1292	0.320	0.0902	2.75
2000	41.1	0.1404	0.328	0.0904	2.93

From Wetzig (2001); Wetzig & Hartmann (2001a)

Table 41. Selected histopathological findings in the dog (n = 5)

Histopathological finding	Incidence (no. of animals with finding)		
	Dietary concentration (ppm)		
	0	100	2000
Adrenal – vacuolation/hypertrophy	0	4	5
Adrenal – mononuclear cell infiltrate	0	1	3
Testes – Leydig cell vacuolation	0	0	5
Testes – hypertrophy	0	0	5
Testes – degeneration of germinal epithelium	0	1	4
Liver – single-cell necrosis	0	0	3
Liver – cytoplasmic change	0	0	3

From Wetzig (2001); Wetzig & Hartmann (2001a)

Concurrently, a minimal to slight degeneration of the testicular germinal epithelium was observed in four high-dose animals. Increased vacuolation in the adrenal cortex was observed in all treated dogs except for one.

Based on the findings in the adrenal gland at both dose levels tested, no NOAEL was established. The LOAEL was the lowest dose tested, 100 ppm (equal to 2.9 mg/kg bw per day). The study authors suggested that the increased LH levels throughout the treatment period, as well as the subsequent Leydig cell hypertrophy, indicated that spirodiclofen causes an activation of the hypothalamic–pituitary–gonadal axis (Wetzig, 2001; Wetzig & Hartmann, 2001a).

Discussion of special studies on mechanisms of endocrine effects

Mechanistic studies were provided that suggested that the enol metabolite (BAJ 2510) may contribute significantly to the effects observed with spirodiclofen. In vitro studies provided evidence that BAJ 2510 disrupted the metabolism of cholesterol, which is a precursor to a variety of hormones. In vitro studies also confirmed that BAJ 2510 could inhibit mitochondrial and cytoplasmic MD in tissue culture, resulting in the reduction of reducing equivalents (in the form of NADPH) required by various cytochrome P450 mono-oxygenases involved in steroidogenesis, the downstream effect of which was ultimately predicted to be reduced hormone production (see Figure 3). In vitro studies

with BAJ 2510, as well as special in vivo studies with spirodiclofen, provided evidence of effects on steroid synthesis. Moreover, lesions observed in the reproductive and endocrine systems in the spirodiclofen database were consistent with perturbations in steroidogenesis. It was proposed by the manufacturer that the Leydig cell and uterine tumours observed in rats were related to these perturbations in steroidogenesis (Freyberger, 2007). Briefly, it was suggested that the inhibition of MD leads to a reduced capacity of cells to provide reducing equivalents necessary for steroid hormone biosynthesis. In response to decreased levels of steroid hormones, there is a prolonged stimulation and increased release of pituitary trophins such as LH. In male rats, it was suggested that such a prolonged stimulation by LH, brought about by a reduction of testosterone-mediated feedback inhibition, was likely responsible for the formation of the Leydig cell tumours observed in male rats. Levels of these hormones were investigated in an 8-week dog study. Treatment with spirodiclofen was associated with increased levels of plasma LH levels; however, decreases in testosterone were not shown. Although an in vitro study provided evidence that testosterone secretion by rat testicular fragments was suppressed by BAJ 2510, testosterone and LH levels were not investigated in the rat in vivo; thus, a direct link between perturbations of these hormones and the development of the Leydig cell tumours could not be made. In female rats, it was suggested that inhibition of ovarian steroid synthesis via the same mechanism involving inhibition of MD results in an imbalance in the estradiol to progesterone ratio, leading to a situation of estrogen dominance, with long-term estrogen stimulation eventually resulting in development of uterine neoplasia. In a 19-week dietary study in female rats, alterations in steroid levels were found to lead to such an altered estrogen to progesterone ratio. However, this effect was noted only at a dose (10 000 ppm; equal to 1209.6 mg/kg bw per day) that was almost 10-fold higher than that at which the uterine tumours were observed (2500 ppm; equal to 152.9 mg/kg bw per day), thus making it difficult to correlate these findings.

The Meeting noted that the Leydig cell and uterine tumours observed in rats are consistent with prolonged perturbations in steroidogenesis and are expected to be threshold mediated. However, a clear description of key events with dose–response relationships and temporal associations was not available, and the Meeting concluded that the data were not sufficient to develop a mode of action for the observed tumours.

3. Studies on metabolites

3.1 Acute toxicity studies

The results of the acute oral as well as dermal and eye irritation studies with metabolites of spirodiclofen are summarized in Tables 42 and 43. All studies complied with GLP.

3.2 Short-term studies of toxicity

Spirodiclofen (purity 98.9%) was administered to groups of Wistar rats (10 of each sex per dose) in the diet at dose levels of 0, 500, 2500 or 5000 ppm (equal to 0, 22.8/26.5, 115/136 and 358/413 mg/kg bw per day [males/females]) for 6 weeks. Additionally, BAJ 2510 (purity 99.5%) was administered to Wistar rats (10 of each sex per dose) in the diet at dose levels of 500, 2500 or 5000 ppm (equal to 37.1/43.2, 184/215 and 372/419 mg/kg bw per day [males/females]). The purpose of this study was to compare the subchronic toxicity of spirodiclofen with that of its enol metabolite (BAJ 2510). Physical examinations were performed weekly. Body weight was recorded once weekly. Food consumption was recorded twice weekly. Selected clinical chemistry parameters (total cholesterol, triglycerides) and hormone (progesterone, corticosterone, aldosterone) levels were determined once during the pretreatment period and at weeks 2, 4 and 6. All animals were necropsied, selected organs were weighed and a range of tissues were taken and fixed. Only the adrenal gland was examined microscopically.

Table 42. Acute oral toxicity of metabolites of spirodiclofen

Metabolite	Purity (%)	Vehicle	Species	Strain	Sex	LD_{50} (mg/kg bw)	Reference
BAJ 2510 (enol)[a]	98.0–99.9	0.5% aqueous carboxymethyl cellulose	Rat	Wistar (HsdCpb:Wu)	M & F	M: 500–1000 F: 300–500	Kroetlinger (2000a)
BAJ 2740-ketohydroxy[b]	98.5	Cremophor EL 2%	Rat	Wistar (HsdCpb:Wu)	M & F	>2500[c]	Kroetlinger (2000b)
BAJ 2740-MA-3-OH-cyclohexyl ester[d]	98.6	Cremophor EL 2%	Rat	Wistar (HsdCpb:Wu)	M & F	>2500[c]	Kroetlinger (2001)

F, female; M, male

[a] Clinical signs following administration included decreased motility in females at ≥200 mg/kg bw and decreased motility and reactivity, digging and cleaning gestures, laboured breathing, increased salivation and uncoordinated gait at ≥500 mg/kg bw. In animals that died, dark red discoloration of the liver and pale discoloration of the spleen were observed at necropsy.

[b] A soil metabolite.

[c] The reported LD_{50} was based on the OECD draft guideline "Acute Oral Toxicity—Acute Toxic Class Method" from 1995. Based on the revised OECD test guideline 423 (adopted in 2001), the company indicated that the interpretation would be as follows: LD_{50} oral >2000 mg/kg bw, equivalent to category "unclassified" of the Globally Harmonized System of Classification and Labelling of Chemicals, and LD_{50} cut-off of >5000 mg/kg bw.

[d] A plant metabolite, also referred to as M05 (2,4-dichloromandelic acid hydroxy-cyclohexyl ester).

Table 43. Summary of dermal and eye irritation studies with BAJ 2510 (enol)

Purity (%)	Route	Concentration (mg)	Species/strain	Sex	Result	Reference
99.9	Dermal	500[a]	Rabbit/ Himalayan	Male	No signs of dermal irritation	Leuschner (2002b)
99.9	Eye	100	Rabbit/ Himalayan	Male	No signs of ocular irritation	Leuschner (2002a)

[a] Test material was pulverized and moistened with water prior to application.

There were no treatment-related mortalities in the study. Treatment-related clinical signs were limited to soiling of the anogenital region or forelimbs, observed with both test substances at the middle and high doses. Body weights were decreased at the high dose (both sexes) with both test substances, and body weight gain per day was dose-dependently decreased at the middle and high doses (both sexes) with both test substances. Overall body weight gain per day was decreased at the middle and high doses for both sexes treated with spirodiclofen, in mid-dose females treated with BAJ 2510 and in high-dose animals of both sexes treated with BAJ 2510. Food consumption was decreased in mid- and high-dose animals treated with both test substances, with the most dramatic decreases occurring during the first week or two of treatment.

For animals treated with spirodiclofen, there were statistically significant increases in mean triglyceride levels (+48%) relative to the control group for females in week 6. The increases observed at weeks 2 and 4 were not considered to be treatment-related changes based on the variations observed in the pretreatment samples. No other treatment-related changes were observed (Table 44). For animals treated with BAJ 2510, although a statistically significant difference was noted for cholesterol concentration in males at 500 ppm in week 2, it was considered to be incidental. Throughout the study, mean triglyceride concentrations were higher in both sexes treated with BAJ 2510 at 5000 ppm and in males at 2500 ppm. Mean total cholesterol concentrations were slightly higher in females at 5000 ppm (both test substances) from week 2 onwards (Table 45). It was noted by the Meeting

Table 44. Triglyceride and cholesterol levels in rats administered spirodiclofen

Week	Mean concentration (mmol/l)[a]							
	Males				Females			
	Dietary concentration of spirodiclofen (ppm)							
	0	500	2500	5000	0	500	2500	5000
Triglycerides								
2	0.65 (—)	0.92 (+42)	0.86 (+32)	0.81 (+25)	0.56 (—)	0.62 (+11)	0.60 (+7)	0.78 (+39)
4	0.66 (—)	0.73 (+11)	0.72 (+9)	0.63 (−5)	0.45 (—)	0.46 (+2)	0.44 (−2)	0.57 (+27)
6	0.65 (—)	0.65 (0)	0.72 (+11)	0.59 (−9)	0.40 (—)	0.41 (+3)	0.39 (−3)	0.59** (+48)
Total cholesterol								
2	1.87 (—)	2.25* (+20)	1.80 (−4)	1.81 (−3)	1.84 (—)	1.98 (+8)	1.81 (−2)	1.99 (+8)
4	1.88 (—)	2.07 (+10)	1.79 (−5)	1.78 (−5)	1.77 (—)	1.95 (+10)	1.79 (+1)	2.10 (+19)
6	1.89 (—)	1.92 (+2)	1.92 (+2)	1.76 (−7)	1.66 (—)	1.87 (+13)	1.92 (+16)	1.99 (+20)

From Blanck (2007)
* $P \leq 0.05$; ** $P \leq 0.01$
[a] Per cent change compared with controls given in parentheses.

Table 45. Triglyceride and cholesterol levels in rats administered BAJ 2510

Week	Mean concentration (mmol/l)[a]							
	Males				Females			
	Dietary concentration of BAJ 2510 (ppm)							
	0	500	2500	5000	0	500	2500	5000
Triglycerides								
2	0.65 (—)	0.95 (+46)	1.24** (+91)	1.26** (+94)	0.56 (—)	0.63 (+13)	0.82 (+46)	1.22** (+118)
4	0.66 (—)	0.65 (−2)	1.10 (+67)	1.18* (+79)	0.45 (—)	0.50 (+11)	0.62 (+38)	0.82** (+82)
6	0.65 (—)	0.67 (+3)	1.13* (+74)	1.06 (+63)	0.40 (—)	0.41 (+3)	0.52 (+30)	0.81** (+103)
Total cholesterol								
2	1.87 (—)	2.10 (+12)	2.04 (+9)	1.97 (+5)	1.84 (—)	1.99 (+8)	2.04 (+11)	2.66** (+23)
4	1.88 (—)	1.89 (+1)	1.91 (+2)	1.83 (−3)	1.77 (—)	1.94 (+10)	1.92 (+8)	2.14* (+21)
6	1.89 (—)	1.83 (−3)	1.87 (−1)	1.75 (−7)	1.66 (—)	1.84 (+11)	1.94 (+17)	1.99* (+20)

From Blanck (2007)
* $P \leq 0.05$; ** $P \leq 0.01$
[a] Per cent change compared with controls given in parentheses.

that in the main toxicology database for spirodiclofen, decreases in cholesterol, often accompanied by decreases in triglyceride levels, were consistently noted in rats after as little as 4 weeks of treatment. The reason for the lack of consistency in these effects in the current study is not clear, but may be related to the use of a different anaesthetic (isoflurane) and the fact that animals were diet-fasted prior to blood sampling in the present study. Isoflurane has been associated with decreases in cholesterol levels in humans and experimental animals, and fasting would also be associated with lower cholesterol levels. It is possible that decreased basal cholesterol levels could mask decreases associated with treatment.

In females dosed with 5000 ppm spirodiclofen, decreases in mean progesterone concentrations from week 2 to week 6 (−40% to −51%), in mean aldosterone concentrations at week 6 (−33%) and in mean corticosterone concentrations at week 6 (−38%) were observed, compared with control values (Table 46). Hormone analysis in animals treated with BAJ 2510 (Table 47) revealed a decrease in mean progesterone concentrations from week 4 to week 6 (approximately −39%) in high-dose females compared with control values. It was noted that interpretation of the progesterone data was limited by the fact that animals were not in the same stage of estrus when blood was sampled.

Effects on the adrenal gland were observed in animals at all dose levels in both test substance groups. These included dose-related increases in absolute and relative weights (at the middle and high doses) (Tables 48 and 49), enlarged and/or pale adrenals (low-dose BAJ 2510 males and mid- and high-dose animals of both sexes treated with both test substances) (Table 50) and dose-related increases in minimal to marked vacuolation of the cortex in the zona fasciculata (Table 51). The adrenal vacuolation tended to be more severe for animals in the BAJ 2510 groups, compared with those treated with spirodiclofen. Other findings noted for mid- and high-dose males (both test

Table 46. Hormone levels in rats administered spirodiclofen[a]

Week	Hormone level (ng/ml or pg/ml)			
	Dietary concentration of spirodiclofen (ppm)			
	0	500	2500	5000
Males				
Progesterone (ng/ml)				
Pretreatment	6.91 ± 2.81	6.54 ± 3.21	6.30 ± 4.42	6.44 ± 2.92
2	3.17 ± 1.81	2.87 ± 1.72	2.81 ± 1.74	3.20 ± 1.11
4	1.68 ± 1.01	1.97 ± 0.92	2.42 ± 1.20	2.86 ± 1.58
6	2.65 ± 1.45	2.46 ± 1.15	2.68 ± 1.40	2.16 ± 0.79
Aldosterone (pg/ml)				
Pretreatment	293.87 ± 84.41	299.61 ± 96.41	254.81 ± 135.57	284.91 ± 92.26
2	166.37 ± 76.00	166.22 ± 95.90	158.20 ± 87.08	196.66 ± 62.51
4	120.63 ± 59.35	150.76 ± 62.36	170.04 ± 83.60	212.10 ± 103.55
6	180.54 ± 118.51	169.43 ± 79.04	171.10 ± 72.18	164.12 ± 56.21
Corticosterone (ng/ml)				
Pretreatment	315.60 ± 96.45	322.73 ± 127.02	260.50 ± 151.26	306.57 ± 114.16
2	195.08 ± 102.45	194.18 ± 114.52	171.13 ± 115.88	244.57 ± 86.64
4	109.09 ± 67.64	146.88 ± 73.66	167.42 ± 92.18	205.44 ± 121.37
6	181.92 ± 114.25	163.24 ± 83.03	187.55 ± 103.53	163.74 ± 76.50
Females				
Progesterone (ng/ml)				
Pretreatment	23.98 ± 5.41	21.38 ± 5.68	23.14 ± 8.64	19.14 ± 7.55
2	27.04 ± 10.69	22.82 ± 8.93	39.54 ± 23.67	16.22 ± 6.49* (−40)[b]
4	27.45 ± 7.28	27.09 ± 7.19	27.22 ± 10.37	13.96 ± 5.50** (−49)[b]
6	34.21 ± 10.90	30.86 ± 8.05	30.55 ± 8.30	16.71 ± 5.30** (−51)[b]

Table 46 (contd)

Week	Hormone level (ng/ml or pg/ml)			
	Dietary concentration of spirodiclofen (ppm)			
	0	500	2500	5000
Aldosterone (pg/ml)				
Pretreatment	471.87 ± 144.99	333.75 ± 135.69	401.97 ± 192.07	352.85 ± 124.96
2	457.74 ± 125.94	433.42 ± 184.85	525.03 ± 161.79	395.62 ± 189.75
4	438.15 ± 131.54	447.32 ± 168.42	471.99 ± 160.81	307.14 ± 136.25
6	400.21 ± 98.34	400.08 ± 167.17	405.27 ± 125.89	266.05 ± 79.49* (−34)[b]
Corticosterone (ng/ml)				
Pretreatment	474.19 ± 144.82	348.38 ± 116.79	415.23 ± 215.54	375.46 ± 166.30
2	495.35 ± 189.41	526.61 ± 314.54	648.76 ± 242.43	507.93 ± 404.77
4	486.29 ± 195.45	618.78 ± 346.11	692.78 ± 388.34	304.58 ± 180.31
6	503.57 ± 129.42	473.62 ± 182.99	547.91 ± 179.70	309.30 ± 107.99** (−39)[b]

From Blanck (2007)
* $P \leq 0.05$; ** $P \leq 0.01$
[a] $n = 9–10$; mean ± standard deviation.
[b] Per cent difference from controls is presented in parentheses.

Table 47. Hormone levels in rats administered BAJ 2510[a]

Week	Hormone level (ng/ml or pg/ml)			
	Dietary concentration of BAJ 2510 (ppm)			
	0	500	2500	5000
Males				
Progesterone (ng/ml)				
Pretreatment	6.91 ± 2.81	5.90 ± 3.01	6.75 ± 3.29	6.63 ± 4.14
2	3.17 ± 1.81	4.37 ± 1.91	3.69 ± 2.16	5.05 ± 2.73
4	1.68 ± 1.01	2.32 ± 1.49	2.15 ± 1.58	3.14 ± 1.90
6	2.65 ± 1.45	2.18 ± 1.70	3.01 ± 0.85	2.71 ± 1.46
Aldosterone (pg/ml)				
Pretreatment	293.87 ± 84.41	278.23 ± 90.64	293.70 ± 96.37	272.35 ± 127.93
2	166.37 ± 76.00	229.41 ± 79.21	218.52 ± 101.51	283.14 ± 124.64
4	120.63 ± 59.35	165.70 ± 94.88	159.83 ± 80.95	259.27 ± 180.22
6	180.54 ± 118.51	153.44 ± 95.93	240.19 ± 89.34	192.71 ± 100.84
Corticosterone (ng/ml)				
Pretreatment	315.60 ± 96.45	304.36 ± 97.14	317.05 ± 112.26	287.91 ± 162.53
2	195.08 ± 102.45	267.46 ± 103.61	241.20 ± 130.79	303.55 ± 137.46
4	109.09 ± 67.64	157.01 ± 104.60	129.33 ± 99.75	194.73 ± 125.17
6	181.92 ± 114.25	143.78 ± 108.07	212.90 ± 72.56	178.86 ± 103.40

Table 47 (contd)

Week	Hormone level (ng/ml or pg/ml)			
	Dietary concentration of BAJ 2510 (ppm)			
	0	500	2500	5000
Females				
Progesterone (ng/ml)				
Pretreatment	23.98 ± 5.41	24.40 ± 6.91	20.08 ± 7.65	21.10 ± 7.32
2	27.04 ± 10.69	27.18 ± 13.36	33.04 ± 21.66	22.78 ± 10.49
4	27.45 ± 7.28	30.03 ± 12.15	25.91 ± 10.12	16.84 ± 3.04* (−39)[b]
6	34.21 ± 10.90	40.09 ± 23.45	32.58 ± 13.46	20.69 ± 10.06* (−40)[b]
Aldosterone (pg/ml)				
Pretreatment	471.87 ± 144.99	460.98 ± 118.45	429.98 ± 126.87	424.19 ± 153.11
2	457.74 ± 125.94	468.67 ± 202.22	551.79 ± 186.62	478.41 ± 201.79
4	438.15 ± 131.54	520.64 ± 219.28	620.56 ± 291.04	373.27 ± 88.14
6	400.21 ± 98.34	449.72 ± 138.51	561.62 ± 388.19	297.57 ± 95.38
Corticosterone (ng/ml)				
Pretreatment	474.19 ± 144.82	450.64 ± 131.94	433.12 ± 147.38	448.60 ± 155.40
2	495.35 ± 189.41	751.62 ± 612.75	817.99 ± 493.10	598.83 ± 405.79
4	486.29 ± 195.45	866.46 ± 602.74	880.93 ± 513.05	413.10 ± 133.82
6	503.57 ± 129.42	515.61 ± 139.85	623.58 ± 393.27	329.50 ± 158.53

From Blanck (2007)
* $P \leq 0.05$
[a] $n = 9–10$; mean ± standard deviation.
[b] Per cent difference from controls is presented in parentheses.

substance groups) included decreased absolute and relative prostate weights and a slight increase in the incidence of atrophic/small prostates and/or seminal vesicles. These organs were not examined histopathologically.

A NOAEL was not established for either spirodiclofen or BAJ 2510. The LOAEL for spirodiclofen was 500 ppm (equal to 22.8 mg/kg bw per day for males and 26.5 mg/kg bw per day for females), based on decreased body weight gain per day and increased incidence of minimal to slight adrenal vacuolation observed in both sexes. The LOAEL for BAJ 2510 was 500 ppm (equal to 37.1 mg/kg bw per day for males and 43.2 mg/kg bw per day for females), based on decreased body weight gain per day and findings in the adrenal gland in both sexes (minimal to slight adrenal vacuolation in both sexes and decreased adrenal weight and increased incidence of paleness in males).

In conclusion, spirodiclofen and its enol metabolite were found to induce similar effects in the rat. There were some indications that the enol metabolite produced more severe effects than the parent compound. For example, the severity of vacuolation in the adrenal cortex was increased slightly in the BAJ 2510 group, indicative of some increase in relative toxicity. However, owing to issues with the stability of spirodiclofen, the adjusted doses of spirodiclofen (on a mg/kg bw per day basis) were lower than those for the enol metabolite. The study author noted that the higher polarity and water solubility of the enol may enhance its bioavailability compared with that of spirodiclofen and may account for some of the differences in comparative toxicity between the compounds. The study author also noted that because of the lower molecular weight of the enol metabolite (313.2) compared

Table 48. Adrenal weights in rats administered spirodiclofen

Parameter	Adrenal gland weight changes at terminal sacrifice[a]							
	Male (n = 10)				Female (n = 10)			
	Dietary concentration of spirodiclofen (ppm)							
	0	500	2500	5000	0	500	2500	5000
Mean absolute adrenal gland weight (g)	0.0577 (—)	0.0623 (+8)	0.0851** (+47)	0.0911** (+58)	0.0695 (—)	0.0717 (+3)	0.0802 (+15)	0.0851** (+22)
Mean adrenal gland to body weight ratio (%)	0.0155 (—)	0.0163 (+5)	0.0232** (+49)	0.0279** (+80)	0.0310 (—)	0.0319 (+3)	0.0363* (+17)	0.0406** (+31)

From Blanck (2007)
* $P \leq 0.05$; ** $P \leq 0.01$
[a] Per cent change compared with controls given in parentheses.

Table 49. Adrenal weights in animals administered BAJ 2510

Parameter	Adrenal gland weight changes at terminal sacrifice[a]							
	Male (n = 10)				Female (n = 10)			
	Dietary concentration of BAJ 2510 (ppm)							
	0	500	2500	5000	0	500	2500	5000
Mean absolute adrenal gland weight (g)	0.0577 (—)	0.0701 (+21)	0.1084** (+88)	0.1323** (+129)	0.0695 (—)	0.0746 (+7)	0.0820 (+18)	0.1037** (+49)
Mean adrenal gland to body weight ratio (%)	0.0155 (—)	0.0185 (+19)	0.0292** (+88)	0.0399** (+157)	0.0310 (—)	0.0333 (+7)	0.0376* (+21)	0.0493** (+59)

From Blanck (2007)
* $P \leq 0.05$; ** $P \leq 0.01$
[a] Per cent change compared with controls given in parentheses.

with that of spirodiclofen (411.4), an equal weight of the enol would represent approximately 1.3 times the number of BAJ 2510 molecules as the parent material, assuming complete conversion in the animal, which is thought to occur on the basis of the metabolism studies. Taken together, these considerations most likely account for the slight difference in response severity noted in the study. Overall, the two compounds were found to exert similar effects in the rat following repeated dosing under the conditions of this study, and it might be concluded that there is no indication in this study that BAJ 2510 produces a different spectrum of toxic effects or causes marked effects at lower doses compared with spirodiclofen (Blanck, 2007).

3.3 Genotoxicity

In vitro reverse mutation studies were conducted with metabolites of spirodiclofen to assess the potential for inducing gene mutation in vitro. All studies complied with GLP. The study results (summarized in the Table 52) were negative. None of the metabolites of spirodiclofen that were tested were found to demonstrate any genotoxic potential under the conditions tested.

3.4 Discussion of metabolites relevant for the dietary risk assessment

The following metabolites were identified only in plants: M05 (2,4-dichloromandelic acid hydroxy-cyclohexyl ester), M04 (2,4-dichloromandelic acid cyclohexyl ester glycosyl pentoside) and

Table 50. Incidence of gross pathology findings in rats

Observation	Incidence (no. of animals with finding)							
	Males (n = 10)				Females (n = 10)			
	Dietary concentration (ppm)							
	0	500	2500	5000	0	500	2500	5000
Spirodiclofen								
Pale adrenal	0	0	3	5	0	0	2	6
Enlarged adrenal	0	0	1	4	1	0	0	1
Seminal vesicles – small and/or atrophic	1	0	2	3	NA	NA	NA	NA
Prostate – small and/or atrophic	0	0	2	2	NA	NA	NA	NA
BAJ 2510								
Pale adrenal	0	3	9	10	0	1	3	10
Enlarged adrenal	0	0	6	9	1	0	1	7
Seminal vesicles – small and/or atrophic	0	0	0	4	NA	NA	NA	NA
Prostate – small and/or atrophic	0	2	0	1	NA	NA	NA	NA

From Blanck (2007)
NA, not applicable

Table 51. Incidence of vacuolation of the zona fasciculata in the adrenal gland of rats

Severity	Incidence (no. of animals with finding)							
	Males (n = 10)				Females (n = 10)			
	Dietary concentration (ppm)							
	0	500	2500	5000	0	500	2500	5000
Spirodiclofen								
Minimal	3	4	0	0	0	2	5	0
Slight	0	1	7	2	0	0	2	9
Moderate	0	0	2	5	0	0	0	1
Marked	0	0	0	3	0	0	0	0
Total	3	5	9	10	0	2	7	10
BAJ 2510								
Minimal	3	3	0	0	0	1	2	0
Slight	0	3	3	0	0	0	7	0
Moderate	0	0	6	1	0	0	0	8
Marked	0	0	1	9	0	0	0	2
Total	3	6	10	10	0	1	9	10

From Blanck (2007)

Table 52. Results of in vitro reverse mutation studies on metabolites of spirodiclofen

Metabolite (purity)	Test object	Concentration	Results	Reference
BAJ 2510 (enol) (98%)[a,b,c]	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	0–5000 µg/plate in DMSO with and without S9 mix	Negative	Herbold (1999)
BAJ 2740-ketohydroxy[d] (98.5%)[a,b,e]	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	0–320 µg/plate (preincubation) with and without S9 mix; in ethanol	Negative	Herbold (2001b)
BAJ 2740-MA-3-OH-cyclohexyl ester[f] (98.6%)[a,b]	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	0–5000 µg/plate in DMSO with and without S9 mix	Negative	Herbold (2001a)

DMSO, dimethyl sulfoxide; S9, 9000 × *g* rat liver supernatant
[a] Positive and negative controls included.
[b] Tested in triplicate, using both the standard plate method and the preincubation method.
[c] Cytotoxicity noted at 5000 µg/plate.
[d] A soil metabolite.
[e] Cytotoxicity noted at ≥500 µg/plate for plate incorporation method; ≥80 µg/plate for preincubation method.
[f] A plant metabolite, also referred to as M05 (2,4-dichloromandelic acid hydroxy-cyclohexyl ester).

M08 (2,4-dichloromandelic acid glucoside). The latter two metabolites are sugar conjugates of minor metabolites found in the rat. Limited toxicology data (acute oral and in vitro reverse mutation studies) were provided for M05, and no toxicology data were provided for the other two plant metabolites. The Meeting therefore concluded that the information available was not sufficient to conduct a hazard assessment for these metabolites. The enol metabolite was detected in plants and livestock matrices. As the enol metabolite was found to be of similar toxicity to the parent compound, the Meeting considered this metabolite to be toxicologically relevant for the dietary risk assessment.

4. Observations in humans

The results from routine medical surveillance of workers in a spirodiclofen production plant were provided for the years 2002–2005. Yearly medical examinations were performed on 90 workers who worked in the plant using the following Bayer CropScience personal protective equipment: full mask with filter (ABEK-P3), protective gloves and chemical-resistant suits. The medical tests included a recording of medical history, full physical examination with orientating neurological status (reflexes, sensibility and coordination) and skin status, as well as the following laboratory and technical examinations: erythrocyte sedimentation rate, full blood count, ASAT, ALAT, γ-glutamyl transferase, glucose, creatinine, cholesterol, urine status, lung function, electrocardiogram/ergometry, vision testing, audiometry, chest X-ray and sonography (if necessary).

Occupational medical surveillance of workers exposed to spirodiclofen, performed every year on a routine basis, not directly related to exposures, did not reveal any unwanted effects in the workers. Since 2002, no accidents with spirodiclofen occurred involving the workers, and no consultations of the medical department due to work or contact with spirodiclofen were required. In addition, no spirodiclofen-related allergenicity observations could be determined during that time period (Kehrig & Steffens, 2005).

Comments

Biochemical aspects

The absorption, distribution, metabolism and excretion of spirodiclofen were investigated in rats. Orally administered ^{14}C-labelled spirodiclofen was rapidly absorbed and eliminated. Blood

concentrations peaked at 3–4 h at low doses (1–2 mg/kg bw) and at ≥8 h at a higher dose (100 mg/kg bw). Decreased urinary excretion at the higher dose suggested saturation of absorption. Urine and faeces were the major routes of excretion. Retention in the carcass and organs was low (total body burden <1% of the administered dose within 48 h), and there was no evidence of bioaccumulation. Dietary pretreatment with non-labelled compound did not have a significant impact on absorption or elimination.

After oral administration, ^{14}C-labelled spirodiclofen was extensively metabolized in rats. Spirodiclofen appears to be rapidly metabolized to the enol metabolite (BAJ 2510). No parent compound was detected in the urine or bile, and up to 11 metabolites were identified, representing 59–90% of the administered dose. Up to 16% of the parent compound was detected in the faeces. The profile of metabolites was generally similar qualitatively in males and females, but varied quantitatively. The major urinary metabolite in females was the enol metabolite, BAJ 2510, whereas the major urinary metabolite in males was the 3-hydroxy-enol metabolite. The metabolic profile in the bile was similar to that observed in the urine; however, a hydroxylated glucuronide metabolite was found to be unique to the bile.

Toxicological data

Spirodiclofen is of low acute toxicity when administered via the oral, dermal and inhalation routes. The oral and dermal LD_{50} values in rats were >2500 and >2000 mg/kg bw, respectively. The inhalation LC_{50} in rats was determined to be >5.03 mg/l air. Spirodiclofen was not irritating to the eyes or skin of rabbits, but was found to be a dermal sensitizer under the conditions of the Magnusson and Kligman maximization test in guinea-pigs.

In short- and long-term studies of oral toxicity in mice, rats and dogs, the primary target organs of toxicity of spirodiclofen were the adrenal glands and testes. The predominant finding was vacuolation of the adrenal cortex, which was noted in the mouse, rat and dog and was often accompanied by increased adrenal weight. With extended duration of dosing, adrenal vacuolation was associated with adrenal hypertrophy in rats and adrenal enlargement and lymphocytic infiltration in mice. In rats, there were no adrenal findings after 4 weeks of dosing (NOAEL of 500 ppm, equal to 50 mg/kg bw per day, on the basis of changes in clinical chemistry parameters and induction of liver enzymes at 5000 ppm), and the overall NOAEL for adrenal effects from the 14-week and 2-year studies was 350 ppm, equal to 14.7 mg/kg bw per day from the 2-year study in rats. In mice, a NOAEL for adrenal findings of 100 ppm, equal to 30 mg/kg bw per day, was identified in the short-term study on the basis of adrenal findings at 1000 ppm in females. After long-term dosing in mice, a NOAEL for adrenal findings could not be identified, as there was an increased incidence of adrenal pigmentation and vacuolation in females at the lowest dose tested (25 ppm, equal to 5.1 mg/kg bw per day). The incidence of pigmentation was only slightly above the reported range for historical controls, and the increase in vacuolation was not statistically significant. However, as the adrenal gland is clearly a target organ, these findings were considered to represent a marginal LOAEL for females at this dose. Compared with other species, the adrenal effects were observed at the lowest doses in dogs. Although a NOAEL could not be established for adrenal findings in the special 8-week study in dogs (LOAEL of 2.9 mg/kg bw per day) or for females in the 14-week study in dogs (LOAEL of 8.4 mg/kg bw per day), an overall NOAEL for adrenal histopathology in the dog was identified from the 1-year study in dogs (NOAEL of 1.4 mg/kg bw per day). Reversibility of the adrenal effects was observed in the short-term study in rats during a 4-week recovery period. The Meeting noted that these adrenal findings are a consequence of repeated dosing, which is supported by the lack of adrenal findings in the 4-week study in rats, and are potentially associated with prolonged perturbation of steroidogenesis.

Testicular effects in mice included Leydig cell hypertrophy and vacuolation after short-term dosing and increased weight, discoloration, degeneration and interstitial cell hyperplasia in the long-term study. In the long-term study, an increase in epididymal aspermia was also noted at the highest dose. In the dog, Leydig cell vacuolation and hypertrophy, testicular immaturity and degeneration

of the germinal epithelium were noted in studies that ranged in duration from 4 weeks to 1 year. In the rat, Leydig cell hyperplasia was observed in the 2-year study. The overall NOAEL for testicular effects was approximately 4 mg/kg bw per day from the 1-year study in dogs (150 ppm), 2-year study in rats (100 ppm) and 18-month study in mice (25 ppm), although it should be noted that increased testes weights were noted at this dose in the 1-year study in dogs. The Meeting noted that when dosing in the dog began at a younger age, effects in reproductive tissues appeared to be more severe, and that the testicular effects were often accompanied by other effects, including immaturity of the prostate and oligospermia/aspermia of the epididymides, which were noted in the 4-week (at 10 000 ppm) and 14-week studies (at ≥630 ppm). The Meeting also observed that there was evidence that effects in male reproductive tissues progressed, and that NOAELs decreased, with increasing duration of exposure, and that the nature of the findings indicated that they were potentially a result of prolonged perturbation of steroidogenesis.

Other effects observed following short- or long-term oral exposure to higher doses of spirodiclofen included effects on the liver, cholesterol levels, thyroid, jejunum and thymus. Liver effects included hypertrophy, vacuolation and hepatocytomegaly in the mouse (at ≥1000 ppm, equal to 164 mg/kg bw per day); necrosis, cytoplasmic change, granulation and inflammatory infiltration in the dog (at ≥2000 ppm, equal to 84.7 mg/kg bw per day); and decreased concentrations of plasma proteins and tigroid basophilic foci in the rat (at 2500 ppm, equal to 110.1 mg/kg bw per day). Increased liver weight and enzyme induction occurring in mice and dogs at lower doses were considered to be an adaptive response to the administration of spirodiclofen. Decreased cholesterol concentrations, which were consistent with the proposed pesticidal mode of action of this chemical, were observed in the rat (at ≥110 mg/kg bw per day), dog (at ≥4 mg/kg bw per day) and mouse (at 1600 mg/kg bw per day) and were accompanied by decreased triglyceride concentrations in the rat. Vacuolation of the jejunum was observed in rats and dogs, and slight atrophy of the thymic cortex was also observed in dogs. Thyroid effects included decreased concentrations of T_4 in the dog (at 2000 ppm in the 4-week study) and an increase in concentrations of TSH (at ≥2500 ppm in the 14-week study) and colloidal alteration of the thyroid (at 2500 ppm in the 2-year study) in rats.

Spirodiclofen was adequately tested for genotoxicity in vitro and in vivo in a range of assays. It was not found to be genotoxic in mammalian or microbial systems.

The Meeting concluded that spirodiclofen was unlikely to be genotoxic.

In an 18-month study of oncogenicity in mice, administration of spirodiclofen at dietary concentrations of 0, 25, 3500 or 7000 ppm resulted in the development of late-onset hepatocellular adenomas and carcinomas in males and females at doses of ≥3500 ppm. Systemic toxicity was noted at the same doses, including changes in organ weights (liver, adrenal gland, testes and kidney) and histopathological findings in the adrenal gland (vacuolation and pigmentation), liver (hepatocytomegaly) and testes (hypertrophy and hyperplasia). As discussed previously, a NOAEL for systemic toxicity was not identified, on the basis of marginal effects on the adrenal gland in females at 25 ppm, equal to 5.1 mg/kg bw per day, the lowest dose tested. The NOAEL for carcinogenicity was 25 ppm, equal to 4.1 mg/kg bw per day, in this study. The Meeting noted that although preneoplastic lesions were not observed at lower doses than those at which the liver tumours were observed, this may have been due to the large dose spacing. Additionally, the Meeting noted that these tumours were observed only at high doses (≥3500 ppm), which also produced hepatotoxicity, and that the dose–response relationship for these tumours was likely to exhibit a threshold.

The toxicity and carcinogenicity of spirodiclofen were investigated in a 2-year dietary study in rats. The incidence of late-onset Leydig cell adenomas was increased in male rats at the highest dose tested (2500 ppm), preceded by an increased incidence of Leydig cell hyperplasia at ≥350 ppm. An increased incidence of uterine adenocarcinomas and uterine nodules was also observed in female rats at the highest dose that had died or were sacrificed before study termination. These adenocarcinomas were noted to have metastasized into various organs of the abdominal cavity, as well

as into the lung and bone marrow. Systemic toxicity was also noted at the highest dose, including increased mortality (females), decreased body weight (by 6–10%), increased levels of AP, decreased concentrations of cholesterol and triglycerides, and histopathological findings in the adrenal gland (vacuolation and hypertrophy; males only), ovary (increased portion of stroma), vagina (possible increase in the number of animals in estrus based on morphology of vaginal epithelium), jejunum (vacuolation), thyroid (colloidal alteration) and olfactory epithelium (atrophy/degeneration; males only). The NOAEL for systemic toxicity was 100 ppm, equal to 4.1 mg/kg bw per day, on the basis of the increased incidence of Leydig cell hyperplasia in males. The NOAEL for carcinogenicity was 350 ppm, equal to 14.7 mg/kg bw per day.

Several special studies were conducted with spirodiclofen and with three of the enol metabolites (BAJ 2510, 3-hydroxy-enol and 4-hydroxy-enol). Studies in vitro provided evidence that the enol metabolite (BAJ 2510) may contribute significantly to the effects observed with spirodiclofen via disruption of the metabolism of cholesterol, which is a precursor to a variety of hormones. Studies also confirmed that BAJ 2510 could inhibit the activity of MD in tissue culture, resulting in a decrease in reducing equivalents required by various cytochrome P450 mono-oxygenases involved in steroidogenesis, the downstream effect of which was ultimately predicted to be reduced hormone production. Studies with BAJ 2510 in vitro, as well as special studies with spirodiclofen in vivo, provided some evidence of effects on steroid synthesis. The Meeting noted that the increased incidence of Leydig cell and uterine tumours observed in rats was consistent with prolonged perturbations in steroidogenesis, and the dose–response relationship for these effects would be anticipated to exhibit a threshold. However, a clear description of key events, with dose–response relationships and temporal associations, was not available, and the Meeting concluded that the data were not sufficient to develop a mode of action for formation of the observed tumours by spirodiclofen.

The Meeting concluded that the relevance of the tumorigenic responses in rats and mice to humans could not be discounted. However, the Meeting noted that spirodiclofen was not genotoxic and that the dose–response relationship for the tumours would be anticipated to exhibit a threshold.

The effect of spirodiclofen on reproduction in rats was investigated in a two-generation study. Parental effects in both generations (F_0 and F_1) included vacuolation of the adrenal cortex and epithelium of the small intestine. Decreases in body weight and in concentrations of cholesterol, triglycerides and unesterified fatty acids were also observed in the F_1 generation (clinical chemistry evaluations were not performed for the F_0 generation). The NOAEL for parental toxicity was 70 ppm, equal to 5.2 mg/kg bw per day. Offspring toxicity included body weight loss and decreased body weight gain in the F_1 and F_2 pups at 350 ppm; the NOAEL for these findings in offspring was 70 ppm, equal to 5.2 mg/kg bw per day. Reproductive toxicity was observed in the F_1 generation only, at the highest dose tested (1750 ppm). Delayed sexual maturation was observed in male offspring, and increased severity of ovarian vacuolation/degeneration, decreased testes, spermatid and epididymal sperm counts, reduced size of testes and epididymides, as well as atrophy of the testes, epididymides and prostate were observed in some F_1 adults at the highest dose. The NOAEL for these findings in F_1 rats was 350 ppm, equal to 26.2 mg/kg bw per day. Although it is possible that the toxic effects on reproduction were associated with exposure in utero (as they were observed in the F_1 generation only), this remains uncertain, considering that F_1 rats began consuming treated diet at an earlier age, experienced a longer duration of dosing and were thus exposed to a higher overall average dose of spirodiclofen than the F_0 generation. The Meeting noted that this reproductive toxicity was potentially caused by sustained alteration of steroidogenesis.

The effect of spirodiclofen on developmental toxicity was investigated in rats and rabbits. In rats, there was no maternal toxicity noted (the NOAEL was 1000 mg/kg bw per day, the highest dose tested), although the Meeting noted that investigation of target organs was not conducted in maternal animals. In the fetus, marginal increases in the incidences of slight renal pelvis dilatation and asymmetrical fourth sternebrae were observed at the highest dose tested. However, as these findings

occurred at the highest dose tested (1000 mg/kg bw per day) and the incidences were within the range for historical controls, the Meeting considered that these effects represented a marginal LOAEL. The Meeting also noted that these findings would not be expected to occur after a single exposure (Solecki et al., 2003; Makris et al., 2009). The NOAEL for developmental toxicity in rats was 300 mg/kg bw per day on the basis of marginal findings at the highest dose tested. In the study in rabbits, maternal toxicity consisted of increased body weight loss and decreased food consumption at 300 mg/kg bw per day. The NOAEL for maternal toxicity in rabbits was 100 mg/kg bw per day, and the NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested.

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

Neurotoxicity was investigated in a study of acute neurotoxicity, a short-term study of toxicity and studies of developmental neurotoxicity in rats. There was no evidence of neurotoxicity in the study of acute neurotoxicity, and the only evidence of neurotoxicity in the short-term study was decreased motor and locomotor activity in females at 12 500 ppm, equal to 1310 mg/kg bw per day (the limit dose), during 1 week of treatment. Two studies of developmental neurotoxicity were conducted. The second was a modified study, intended to clarify potential findings related to brain morphometry and learning and memory parameters in offspring in the first study. Effects in maternal animals were limited to small changes in body weight and/or food consumption at the highest dose tested, and these effects were not considered to be biologically relevant. The NOAEL for maternal toxicity in both studies was 1500 ppm, equal to 119 mg/kg bw per day, the highest dose tested. In offspring, observed morphometric changes were small (3–7%), did not attain statistical significance in many cases and were not consistent between studies, and thus they were not considered to be related to treatment. In tests of learning and memory, the findings were also inconsistent, and the considerable variability in the data limited their interpretation. Overall, the Meeting considered that these studies did not indicate any treatment-related findings on neurotoxicity parameters in offspring. The NOAEL was 350 ppm, equal to 28.6 mg/kg bw per day, on the basis of decreases in body weight and body weight gain in offspring at 1500 ppm.

Studies of acute toxicity, short-term studies of toxicity and studies of genotoxicity were conducted with some of the metabolites of spirodiclofen, including BAJ 2740-ketohydroxy (a soil metabolite) and BAJ 2740-MA-3-OH-cyclohexyl ester (a plant metabolite)—neither of which were detected in the studies of metabolism in rats——as well as the enol metabolite (BAJ 2510). BAJ 2740-ketohydroxy and BAJ 2740-MA-3-OH-cyclohexyl ester were both of low acute toxicity when administered orally. BAJ 2510 was moderately toxic via the oral route (LD_{50}, 300–500 mg/kg bw per day) and was not irritating to the eyes or skin. A 6-week dietary study comparing the relative toxicities of spirodiclofen and the enol metabolite was conducted. Effects noted with both test substances included decreased body weight and food consumption, increased adrenal weights, and enlargement and vacuolation of the adrenal gland. Both were also associated with decreased progesterone concentrations in females. In this short-term study, spirodiclofen and the enol metabolite were found to exert similar effects in rats given repeated doses under the conditions of the study, and there was no indication that BAJ 2510 produced a different spectrum of toxic effects or caused marked effects at lower doses than did spirodiclofen. Studies of reverse mutation in vitro were conducted with three metabolites of spirodiclofen (BAJ 2510, BAJ 2740-ketohydroxy and BAJ 2740-MA-3-OH-cyclohexyl ester) to assess potential for inducing gene mutation in vitro. None of these metabolites of spirodiclofen were found to demonstrate any mutagenic potential under the conditions tested.

The following metabolites were identified only in plants: M05 (2,4-dichloromandelic acid hydroxy-cyclohexyl ester), M04 (2,4-dichloromandelic acid cyclohexyl ester glycosylpentoside) and M08 (2,4-dichloromandelic acid glucoside). The latter two metabolites are sugar conjugates of minor metabolites found in the rat. Limited toxicology data were provided for M05, and no toxicology data were provided for the other two plant metabolites. The Meeting therefore concluded that the information available was not sufficient to conduct a hazard assessment for these metabolites. The enol

metabolite was detected in plants and livestock matrices. As the enol metabolite was found to be of similar toxicity to the parent compound, the Meeting considered this metabolite to be toxicologically relevant for the dietary risk assessment.

There were no reports of adverse health effects in manufacturing plant personnel or in operators and workers exposed to spirodiclofen formulations.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.01 mg/kg bw per day based on the NOAEL of 1.4 mg/kg bw per day identified on the basis of adrenal effects in both sexes and increased relative testes weights in males at 4.3 mg/kg bw per day in the 1-year study in dogs, and with a safety factor of 100. This ADI provides adequate protection for the marginal adrenal effects noted in females at the lowest dose in the 18-month study in mice. The ADI provides a margin of at least 410-fold relative to the NOAEL for liver tumours in mice and 1470-fold relative to the NOAEL for Leydig cell and uterine tumours in rats, and thus the Meeting considered that spirodiclofen was not likely to pose a carcinogenic risk to humans at dietary levels of exposure.

The Meeting noted that spirodiclofen was not acutely toxic after short-term dosing, that there were no adverse findings in a study of acute neurotoxicity and that there were no developmental toxicity findings that were expected to occur after a single dose in studies in rats or rabbits. The Meeting also noted that findings in the male reproductive system (observed in dogs, rats and mice) would not be caused by a single dose. Consequently, the Meeting determined that an acute reference dose (ARfD) was unnecessary.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	—	25 ppm, equal to 5.1 mg/kg bw per day[b]
		Carcinogenicity	25 ppm, equal to 4.1 mg/kg bw per day	3500 ppm, equal to 610 mg/kg bw per day
Rat	Two-year studies[a]	Toxicity	100 ppm, equal to 4.1 mg/kg bw per day	350 ppm, equal to 14.7 mg/kg bw per day
		Carcinogenicity	350 ppm, equal to 14.7 mg/kg bw per day	2500 ppm, equal to 110.1 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Parental toxicity	70 ppm, equal to 5.2 mg/kg bw per day	350 ppm, equal to 26.2 mg/kg bw per day
		Reproductive toxicity	350 ppm, equal to 26.2 mg/kg bw per day	1750 ppm, equal to 134.5 mg/kg bw per day
		Offspring toxicity	70 ppm, equal to 5.2 mg/kg bw per day	350 ppm, equal to 26.2 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	1000 mg/kg bw per day[e]	—
		Embryo/ fetotoxicity	300 mg/kg bw per day	1000 mg/kg bw per day
	Developmental neurotoxicity[a,d]	Maternal toxicity	1500 ppm, equal to 119 mg/kg bw per day[e]	—
		Offspring toxicity	350 ppm, equal to 28.6 mg/kg bw per day	1500 ppm, equal to 119 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
Rabbit	Developmental toxicity[c]	Maternal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Embryo/ fetotoxicity	1000 mg/kg bw per day[e]	—
Dog	Eight-week study of toxicity[a,f]	Toxicity	—	100 ppm, equal to 2.9 mg/kg bw per day[b]
	Fourteen-week study of toxicity[a]	Toxicity in males	200 ppm, equal to 7.7 mg/kg bw per day	630 ppm, equal to 26.6 mg/kg bw per day
		Toxicity in females	—	200 ppm, equal to 8.4 mg/kg bw per day[b]
	One-year study of toxicity[a]	Toxicity	50 ppm, equal to 1.4 mg/kg bw per day	150 ppm, equal to 4.3 mg/kg bw per day

[a] Dietary administration.
[b] Lowest dose tested.
[c] Gavage administration.
[d] Two studies combined.
[e] Highest dose tested.
[f] Study conducted with males only.

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

Unnecessary

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid (T_{max} of 3–4 h) and extensive (up to 76%; based on renal excretion data)
Distribution	Widely distributed; highest concentrations in liver and kidneys
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapidly excreted; 90% eliminated at 48 h
Metabolism in animals	Extensively metabolized; no parent compound detected in urine or bile. Quantitative differences in metabolic profile between sexes.
Toxicologically significant compounds (animals, plants, environment)	Parent compound and enol metabolite
Acute toxicity	
Rat, LD_{50}, oral	>2500 mg/kg bw
Rat, LD_{50}, dermal	>2000 mg/kg bw
Rat, LC_{50}, inhalation	>5.03 mg/l air
Rabbit, dermal irritation	Not an irritant
Rabbit, ocular irritation	Not an irritant
Guinea-pig, dermal sensitization	Sensitizing (maximization test)

Short-term studies of toxicity	
Target/critical effect	Adrenal gland (cortical vacuolation and increased weight)
Lowest relevant oral NOAEL	50 ppm (equal to 1.4 mg/kg bw per day; 1-year study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (28-day study in rats)
Genotoxicity	
	No genotoxic potential
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Adrenal gland (cortical vacuolation and increased weight)
Lowest relevant NOAEL	50 ppm (equal to 1.4 mg/kg bw per day; 1-year study in dogs)
Carcinogenicity	Tumours in livers (mice), testes (rat) and uterus (rat) at doses that caused target organ and/or systemic toxicity. NOAELs identified; unlikely to pose a risk to humans at levels of dietary exposure.
Reproductive toxicity	
Reproduction target/critical effect	Delayed sexual maturation, decreased spermatid and sperm counts, atrophy of male sex organs, and ovarian vacuolation/degeneration in F_1 animals
Lowest relevant reproductive NOAEL	350 ppm (equal to 26.2 mg/kg bw per day)
Developmental target/critical effect	Renal pelvis dilatation and asymmetric fourth sternebrae
Lowest relevant developmental NOAEL	300 mg/kg bw per day (rat)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	No evidence of neurotoxicity; NOAEL: 1000 mg/kg bw per day
Subchronic neurotoxicity	Decreased motor and locomotor activity (females only); NOAEL: 87 mg/kg bw per day
Developmental neurotoxicity	No evidence of developmental neurotoxicity
Other toxicological studies	
Mechanism studies	Possible effect on steroidogenesis by the enol metabolite via effects on MD
Studies with metabolites	
Acute toxicity	BAJ 2510 was moderately acutely toxic via the oral route (LD_{50} 300–500 mg/kg bw); BAJ 2740-ketohydroxy and BAJ 2740-3-OH-cyclohexyl ester were of low acute oral toxicity (LD_{50} >2500 mg/kg bw)
Short-term toxicity	Similar results following short-term dosing with BAJ 2510 and spirodiclofen: decreased body weight and adrenal effects
Genotoxicity	BAJ 2510, BAJ 2740-ketohydroxy and BAJ 2740-3-OH-cyclohexyl ester were not mutagenic in vitro
Medical data	
	No occupational or accidental poisoning reported

Summary

	Value	Study	Safety factor
ADI	0–0.01	Dog, 1-year	100
ARfD	Unnecessary	—	—

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ANNEX 1

Reports and other documents resulting from previous Joint Meetings of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues

1. Principles governing consumer safety in relation to pesticide residues. Report of a meeting of a WHO Expert Committee on Pesticide Residues held jointly with the FAO Panel of Experts on the Use of Pesticides in Agriculture. FAO Plant Production and Protection Division Report, No. PL/1961/11; WHO Technical Report Series, No. 240, 1962.

2. Evaluation of the toxicity of pesticide residues in food. Report of a Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues. FAO Meeting Report, No. PL/1963/13; WHO/Food Add./23, 1964.

3. Evaluation of the toxicity of pesticide residues in food. Report of the Second Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues. FAO Meeting Report, No. PL/1965/10; WHO/Food Add./26.65, 1965.

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This volume contains toxicological monographs that were prepared by the 2009 Joint FAO/WHO Meeting on Pesticide Residues (JMPR), which met in Geneva on 16–25 September 2009.

The monographs in this volume summarize the safety data on eight pesticides that could leave residues in food commodities. These pesticides are bifenthrin, cadusafos, chlorothalonil, chlorpyrifos-methyl, cycloxydim, fluopicolide, metaflumizone and spirodiclofen. 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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