Pesticide residues in food – 2006

Joint FAO/WHO Meeting on Pesticide Residues

EVALUATIONS 2006

Part II – Toxicological

IPCS International Programme on Chemical Safety

Food and Agriculture Organization of the United Nations

World Health Organization

Pesticide residues in food — 2006

Toxicological evaluations

Sponsored jointly by FAO and WHO
With the support of the International Programme on Chemical Safety (IPCS)

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

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Rome, 3–12 October 2006

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Abbreviations used

ADI	acceptable daily intake
ALT	alanine aminotransferase
APDM	aminopyrine *N*-demethylase
ARfD	acute reference dose
AST	aspartate aminotransferase
AUC	area under the curve of concentration–time
bw	body weight
CAS	Chemical Abstracts Service
CAT	carnitine acyl transferase
Cl	clearance
CMC	carboxymethyl cellulose
DMSO	dimethylsulfoxide
ECG	electrocardiogram
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
FAOX	fatty acid beta-oxidation
FCA	Freund complete adjuvant
FOB	functional observational battery
GCMS	gas chromatography-mass spectrometry
GLP	good laboratory practice
GPD	glycerophosphate dehydrogenase
GSD	geometric standard deviation
HPLC	high-performance liquid chromatography
HPLC-UV	high-performance liquid chromatography with ultraviolet light detector
IPCS	International Programme on Chemical Safety
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
LC_{50}	median lethal concentration
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOQ	limit of quantification
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MMAD	mass median aerodynamic diameter
MoE	margin of exposure

MRL	maximum residue level
MRT	mean residence time
MS	mass spectrometry
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NMR	nuclear magnetic resonance
OECD	Organisation for Economic Co-operation and Development
ppm	parts per million
PAM	pyridine-2-aldoxime methane sulfonate
QA	quality assurance
SER	smooth endoplasmic reticulum
T3	triiodothyronine
T4	thyroxin
TLC	thin-layer chromatography
TMDI	theoretical maximum daily intake
TOCP	tri-*ortho*-cresyl phosphate
TPO	thyroid peroxidase
TRR	total radiolabelled residue
TSH	thyroid-stimulating hormone
Vd	volume of distribution
WHO	World Health Organization
w/v	weight for volume
w/w	weight for weight

Introduction

The toxicological monographs and monograph addenda contained in this volume were prepared by a WHO Core Assessment Group that met with the FAO Panel of Experts on Pesticide Residues in Food and the Environment in a Joint Meeting on Pesticide Residues (JMPR) in Rome, Italy, on 3–12 October 2006.

The Meeting evaluated 30 pesticides, including five new compounds and five compounds that were re-evaluated within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR) for toxicity or residues, or both. The original schedule of compounds to be evaluated was amended for cypermethrins and triadimefon/triadimenol. For cypermethrins, only a toxicological evaluation was carried out as no residue data were received. The evaluation of triadimefon/triadimenol was postponed to 2007 as the peer review process could not be completed in time. 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 187*. 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 2006, Part I, Residues*, in the FAO Plant Production and Protection Paper series.

The toxicological monographs and addenda contained in this volume are based on working papers that were prepared by temporary advisers before the 2006 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.

The preparation and editing of this volume was made possible by the technical and financial contributions of the lead institutions of the International Programme on Chemical Safety (IPCS), which supports the activities of the JMPR. The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Central Unit of the IPCS concerning the legal status of any country, territory, city or area or of its authorities, nor concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the IPCS in preference to others of a similar nature that are not mentioned.

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

TOXICOLOGICAL MONOGRAPHS AND MONOGRAPH ADDENDA

BIFENAZATE

First draft prepared by
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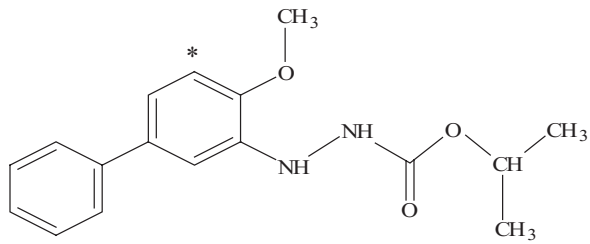
Explanation

Bifenazate is the International Organization of Standardization (ISO) approved name for *N*-(4-methoxy-biphenyl-3-yl) hydrazine-carboxylic acid isopropyl ester (International Union of Pure and Applied Chemistry). Bifenazate, a carbazate compound, is a new acaricide with a unique mode of action that is very selective for spider mites. It is intended for use on apples, pears, nectarines, peaches, plums, prunes, strawberries, grapes, hops and ornamentals.

Bifenazate was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) at the request of the Thirty-eighth Session of the Codex Committee on Pesticide Residues (CCPR). The Meeting has not previously evaluated bifenazate.

All pivotal studies with bifenazate were certified as complying with good laboratory practice (GLP).

Figure 1. Chemical structure of bifenazate



* Position of the bifenazate radiolabel used in the pharmacokinetic studies in rats

Evaluation for acceptable daily intake

1. Biochemical aspects: absorption, distribution, and excretion

Rats

The position of the radiolabel on bifenazate used in absorption, distribution, metabolism and excretion studies is shown in Figure 1.

In a dose range-finding study, groups of two male and two female fasted Sprague-Dawley rats were treated with [methoxyphenyl U-ring ^{14}C]bifenazate (lot No. CSL-94-516-73-20; purity, > 98%) in corn oil at 10 or 1000 mg/kg bw by gavage. Rats were housed individually in glass metabolism cages with food and water freely available. Dose preparations were analysed for chemical concentration and radiochemical purity by high pressure liquid chromatography (HPLC). Expired air from each animal passed through two trapping vessels that contained 40% (w/v) potassium hydroxide (KOH) and water (to trap carbon dioxide) and one trapping tube that contained Chromosorb (to trap radiolabelled volatile organic compounds). The trapping vessels were placed in a dry-ice bath. Expired air, urine and faeces were collected at intervals after dosing for 168 h, with cage washes done at the same intervals. At the end of the collection period, rats were killed and blood collected. Carcasses were weighed, homogenized and analysed. All samples were analysed for radioactivity by liquid scintillation counting (LSC).

The recovery of radiolabel was 93.77% and 100.85% of the administered dose of 10 and 1000 mg/kg bw, respectively. Radioactivity in the expired air was 0.21% and 0.6% of the 10 and 1000 mg/kg bw doses, respectively. Excretion via urine made up 12.4% of the dose at 10 mg/kg bw and 4.79% at 1000 mg/kg bw. Urinary excretion was 96% complete after 72 h at 10 mg/kg bw, and 61% complete after 72 h at 1000 mg/kg bw. Faecal excretion accounted for 68.9% of the administered dose at 10 mg/kg bw, and 91.6% at 1000 mg/kg bw. At 10 mg/kg bw, faecal excretion was 95% complete after 48 h, and at 1000 mg/kg, 61% complete at 72 h. Overall, more than 90% of excretion (via all routes) was complete after 72 h at 10 mg/kg bw, and after 96 h at 1000 mg/kg bw. At 168 h after dosing, blood accounted for 0.13% of the dose at 10 mg/kg bw and 0.14% at 1000 mg/kg bw. Concentrations in the carcass were 0.55% of the dose at 10 mg/kg bw and 0.33% of the dose at 1000 mg/kg bw (Andre & McClanahan, 1997).

In a series of experiments, groups of five male and five female Sprague-Dawley rats were given single oral doses of [methoxyphenyl U-ring ^{14}C]bifenazate (lot No. CSL-94-516-73-20; purity, > 98%) at 10 or 1000 mg/kg bw in corn oil. The administration volume was 10 ml/kg bw for oral dosing. Treated rats were housed individually in glass metabolism cages with food and water freely available. Urine and faeces were collected at intervals of 6, 12, 24, 48, 72, 96, 120, 144 and 168 h after dosing. Expired air was not collected since it was not shown to be a significant route of elimination in the pilot study. At the termination of the study, samples of brain, heart, lungs, liver, adrenals, kidneys, gastrointestinal tract and contents, bone (femur), bone marrow, muscle, mesenteric fat, mesenteric lymph nodes, testes, ovaries, seminal vesicle (uterus), urinary bladder, eyeballs, pituitary, mandibular salivary glands, spleen, pancreas, thyroid, thymus, whole blood, plasma and erythrocytes were collected and analysed for radioactivity. The remaining carcass and cage washings were also analysed for radioactivity.

Overall recovery of administered radioactivity was excellent and was approximately 93–98% for the single-dose, single highest-dose (Table 1), and biliary studies. Most of the dose was excreted in the faeces and to a lesser extent, in the urine. In rats at 10 mg/kg bw, approximately 24% and 66% of the administered dose was excreted in the urine and faeces, respectively. At the highest dose of 1000 mg/kg bw, greater amounts were excreted in the faeces (82.0–82.8%) with less excreted in the urine (7.9% and 9.4% for males and females, respectively). A small amount of radioactivity (up to 5.2% of the administered dose) was recovered in the cage wash. At 10 mg/kg bw, 90% of the urinary and faecal excretion was complete by 48 h after dosing (Table 2). At 1000 mg/kg bw, 90% of the urinary and faecal excretion was complete by 96 h after dosing (Table 3). At 1000 mg/kg bw, males excreted more radioactivity into urine and faeces than did females (77% vs 54%) by 48 h.

Table 1. Mean recovery of radioactivity (as a percentage of administered dose ± standard deviation) at 16 h after in rats given radiolabelled bifenazate as a single dose by oral gavage

Samples	Administered dose (mg/kg bw)[a]			
	10		1000	
	Males	Females	Males	Females
Faeces	66.1 ± 3.9	66.4 ± 3.0	82.0 ± 6.3	82.8 ± 3.3
Urine	24.3 ± 3.1	24.7 ± 4.0	7.9 ± 1.3	9.4 ± 1.0
Cage wash	3.3 ± 0.6	4.1 ± 1.2	3.3 ± 2.1	5.2 ± 1.3
Tissues	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Carcass	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
Total	94.3 ± 2.8	95.9 ± 4.0	93.6 ± 5.7	97.9 ± 2.1

From McClanahan (1998)

[a] n = 5 for all groups

Table 2. Mean cumulative excretion of radioactivity (as a percentage of dose) in urine and faeces of rats given radiolabelled bifenazate at a dose of 10 mg/kg bw by oral gavage

Time-point (h)	Males[a]				Females[a]			
	Urine	Faeces	Cage wash	Total	Urine	Faeces	Cage wash	Total
6	1.9	NS	0.7	2.6	4.7	NS	1.7	6.3
12	10.7	NS	1.7	12.5	1.8	NS	2.9	14.7
24	19.7	54.1	2.7	76.5	20.2	49.5	3.8	73.5
48	23.4	63.5	3.1	90.0	23.8	62.5	4.0	90.3
72	24.0	65.1	3.2	92.4	24.5	65.3	4.1	93.9
96	24.2	65.6	3.3	93.0	24.6	65.9	4.1	94.6
120	24.3	65.8	3.3	93.4	24.6	66.2	4.1	94.9
144	24.3	65.9	3.3	93.4	24.7	66.3	4.1	95.1
168	24.3	66.1	3.3	93.7	24.7	66.4	4.1	95.2

From McClanahan (1998)
NS, no sample.
[a] $n = 5$ for all groups.

Table 3. Mean cumulative excretion of radioactivity (as a percentage of administered dose) in urine and faeces of rats given radiolabelled bifenazate at a dose of 1000 mg/kg bw by oral gavage

Time-point (h)	Males[a]				Females[a]			
	Urine	Faeces	Cage wash	Total	Urine	Faeces	Cage wash	Total
6	0.3	NS	0.1	0.4	0.4	NS	0.3	0.7
12	0.8	NS	0.4	1.1	0.8	NS	0.8	1.6
24	2.4	47.0	1.6	51.1	2.1	32.3	1.8	42.2
48	5.5	68.9	2.6	76.9	4.6	45.5	3.7	53.9
72	7.2	78.2	3.0	88.4	7.9	74.6	4.8	87.3
96	7.7	81.0	3.1	91.7	8.9	80.5	5.1	94.5
120	7.8	81.7	3.2	92.7	9.2	82.3	5.2	96.7
144	7.9	81.9	3.3	93.0	9.3	82.7	5.2	97.2
168	7.9	82.0	3.3	93.2	9.4	82.8	5.2	97.4

From McClanahan (1998)
NS, no sample.
[a] $n = 5$ for all groups.

After 168 h, total residues in the tissues and carcass were < 0.6% of the administered dose. The highest concentrations of radioactivity were found in the liver, kidney, whole blood, heart, spleen, erythrocytes and lungs (Table 4). There was no difference in tissue residue concentrations between males and females at 10 mg/kg bw. In animals at 1000 mg/kg bw, females had higher residue concentrations than males in the liver, kidney, heart, spleen and erythrocytes. The difference was most evident in erythrocytes and the spleen.

In a study of the time-course of distribution, three male and three female rats per time-point were given radiolabelled bifenazate as a single dose at 10 or 1000 mg/kg bw and sacrificed at 6, 24 h or 48 h after dosing (10 mg/kg bw) or at 18, 42 or 72 h after dosing (1000 mg/kg bw). The highest concentrations were typically seen in the liver, kidney, whole blood, heart, spleen and erythrocytes, while the lowest were in the adrenals, eyes, muscle, pituitary, bone marrow and seminal vesicles or uterus. At 10 mg/kg bw, concentrations in all tissues decreased over the observation period, and were generally lower in females than in males. At 1000 mg/kg bw, concentrations peaked at 42 h, with a slower decline from erythrocytes than from most other tissues. Spleen concentrations also increased for longer than in other tissues. The erythrocyte and spleen effects were more prominent in females than males. While initially concentrations were lower in females, concentrations at 72 h were either similar or higher in females than in males. No toxicologically significant differences in the tissue concentrations were apparent between the sexes at either 10 or 1000 mg/kg bw.

Table 4. Mean residues in selected tissues of rats (mg of bifenazate equivalents/kg and percentage of administered dose) in rats given a single dose of radiolabelled bifenazate by oral gavage

Tissue	Males[a]				Females[a]			
	10 mg/kg bw		1000 mg/kg bw		10 mg/kg bw		1000 mg/kg bw	
	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
Gastrointestinal tract + contents	0.029	0.04	1.771	0.03	0.028	0.04	3.451	0.05
Liver	0.400	0.21	11.116	0.06	0.421	0.21	18.037	0.10
Kidney	0.195	0.02	10.782	0.01	0.204	0.02	14.617	0.02
Whole blood	0.135	0.11	15.375	0.12	0.166	0.13	14.817	0.11
Heart	0.068	< 0.005	4.863	< 0.005	0.072	< 0.005	7.883	< 0.005
Spleen	0.056	< 0.005	25.255	0.01	0.072	< 0.005	68.243	0.04
Erythrocytes	0.209	0.07	28.926	0.09	0.270	0.08	47.163	0.13
Lung	0.067	< 0.005	4.490	< 0.005	0.089	0.01	6.080	< 0.005
Thyroid	0.013	ND	ND	ND	ND	ND	ND	ND
Total (all tissues)[b]	—	0.4	—	0.24	—	0.41	—	0.32
Residual carcass	0.02	0.20	1.94	0.17	0.03	0.24	2.89	0.18

From McClanahan (1998)
ND, not detected.
[a] $n = 5$ for all groups
[b] Not including erythrocytes, blood plasma, mesenteric fat, bone and muscle.

In another experiment, groups of three male and three female Sprague-Dawley rats were given radiolabelled bifenazate as a single dose at 10 or 1000 mg/kg bw to investigate biliary elimination and the time-course of tissue distribution. In bile-duct cannulated animals, bile was collected at 1, 2, 4, 6, 8, 10, 12, 16, 24, 48 and 72 h after dosing, urine at 6, 12, 24, 48 and 72 h after dosing and faeces at 24, 48 and 72 h after dosing. The gastrointestinal tract of these animals was excised and analysed for radioactivity at the end of the experiment.

In the bile-duct cannulation experiment at 10 mg/kg bw, approximately 7% of the administered dose was excreted in the faeces, 11% in the urine and 68–73% in the bile. Residual concentrations in the carcass were approximately 1.4% of the administered dose, and concentrations in the gastrointestinal tract were 0.17% in males and 0.81% in females. At 1000 mg/kg bw in males, 57% of the administered dose was excreted in the faeces, 3.4% in the urine and 26% in the bile, while in females 64.2% of the administered dose was excreted in the faeces, 1.4% in the urine and 21% in the bile. Approximately 1% of the administered dose remained in the carcass, with 5% in the gastrointestinal tract of males and 8% in the gastrointestinal tract in females. In bile-duct cannulated rats, cumulative excretion indicated that > 87% of the administered dose had been excreted by 72 h at both doses. The study author estimated that, based on urinary and biliary excretion at 10 mg/kg bw, approximately 85% of the administered dose was absorbed in males and 79% in females, while at 1000 mg/kg bw, approximately 29% of the administered dose was absorbed in males and 22% in females. Absorption at 1000 mg/kg bw appeared to have reached saturation; absorption in males was slightly higher than in females.

The pharmacokinetics of radiolabelled bifenazate were studied by determining the concentration of radioactivity in blood samples. Samples were taken from rats at 10 mg/kg bw at 0, 2.5, 4, 5, 6, 8, 10, 12, 24, 36, 48, 72 and 96 h after dosing and from rats at 1000 mg/kg bw at 1, 3, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72, 96, 120 and 144 h after dosing.

The pharmacokinetic parameters were calculated using non-compartmental analysis (Table 5). The maximum plasma concentration (C_{max}) was reached earlier after a dose of 10 mg/kg bw (5–6 h) than after a dose of 1000 mg/kg bw (18–24 h). However, the elimination half-lives were slightly longer at 1000 mg/kg bw (12–16 h) than at 10 mg/kg bw (12–13 h). No apparent sex-related differences in the pharmacokinetics of bifenazate were observed. The relative systemic exposure to the test material by comparison of the plasma C_{max} and the area under the curve (AUC) shows dose–dependency and is consistent with saturation of absorption at 1000 mg/kg bw (McClanahan, 1998).

Table 5. Pharmacokinetic parameters based on total radiolabel in rats given radiolabelled bifenazate as a single dose by oral gavage

Parameter	Dose (mg/kg bw)			
	10		1000	
	Male	Female	Male	Female
T_{max}	5 h	6 h	18 h	24 h
C_{max}	6.37 mg/kg	5.58 mg/kg	119.0 mg/kg	71.35 mg/kg
AUC	121 µg·h/g	79 µg·h/g	5909 µg·h/g	4733 µg·h/g
$T_{½}$	11.5 h	13.3 h	12 h	15.6 h

From McClanahan (1998)
AUC, area under the curve of concentration–time.

In a repeat-dose pharmacokinetic study, groups of five male and five female Sprague-Dawley rats were treated with 14 consecutive daily oral doses of unlabelled bifenazate at 10 mg/kg bw per day, followed by a single oral dose of [methoxyphenyl U-ring ^{14}C]labelled bifenazate at a concentration of 10 mg/kg bw in corn oil. Urine and faeces were collected over dry ice at 24-h intervals after administration of the final dose, with sacrifice at 168 h; samples of selected tissues, carcass and cage washings were taken for analysis.

Approximately 90% of the administered dose was excreted. The extent of excretion was similar in males and females. At 168 h after dosing, approximately 34% and 30% of the administered dose was excreted in the urine of males and females respectively, with most (approximately 75%) being excreted in the first 24 h. Radioactivity recovered in the faeces accounted for approximately 54% and 57% of

the administered dose in males and females, respectively. Less than 0.5% of the administered dose remained in the body. Approximately 0.13% of the administered dose was found in the liver. There were no differences in tissue residue concentrations between males and females (Banijamali, 2001).

Samples of excreta collected for 96 h after dosing from the single-dose study were analysed to identify metabolites. Samples from three out of five animals per group were pooled by sex and subjected to extraction and chromatographic analysis. Excreta from three animals of each sex per dose from the biliary excretion study were pooled for the 72 h after dosing. The structures of the metabolites were determined by several methods, including enzymatic or chemical degradation. Excreta from the repeat-dose study collected at 24 h and 48 h after dosing (five animals of each sex) were pooled by sex and collection time, separately.

Table 6. Distribution of metabolites (as a percentage of administered radioactivity) after oral administration of radiolabelled bifenazate to bile-cannulated rats

Metabolites[a]	Single oral dose study				Bile-cannulation study			
	10 mg/kg bw		1000 mg/kg bw		10 mg/kg bw		1000 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine[b]								
Urine sample	25.38	23.92	8.47	9.40	11.30	10.61	3.36	1.42
Identified	20.76	18.59	7.44	5.81	4.08	4.58	1.81	0.84
D9569 conjugate	4.83	0.17	0.73	ND	0.43	0.30	0.36	0.13
D9569 sulfate	11.76	8.97	2.27	0.45	3.32	2.59	0.49	0.15
A1530 sulfate	4.17	9.45	4.44	5.36	0.34	1.70	0.96	0.56
Unidentified	4.54	5.23	0.98	3.59	6.66	5.66	1.33	0.20
Faeces[c]								
Faecal sample	64.18	66.25	84.22	79.31	7.40	7.94	56.72	64.59
Extractable	47.88	50.91	74.95	68.33	6.19	5.73	60.11	60.35
Identified	39.29	39.94	71.64	64.64	4.43	3.64	58.27	58.09
D2341 glucuronide	8.92	6.3	5.63	4.71	—	—	—	—
D9569	1.75	0.93	0.15	0.17	—	—	—	—
4-OH D2341	3.56	6.77	2.37	6.64	—	—	—	—
D9477	2.27	2.98	0.05	1.21	—	—	—	—
A1530	5.54	7.06	1.19	2.07	—	—	—	—
4-OH D3598	2.40	5.56	ND	0.23	—	—	—	—
D2341	7.21	4.82	61.26	47.88	2.70	1.32	57.03	56.11
D1989	3.49	0.51	0.11	0.12	1.08	1.37	0.41	1.44
D3598	4.15	5.01	0.88	1.61	0.66	0.95	0.83	0.10
Unidentified	8.46	10.81	3.06	3.22	1.43	1.32	ND	ND
Unextractable	14.61	14.92	10.20	11.61	1.66	2.05	2.59	1.66
Bile[d]								
Bile sample	—	—	—	—	71.86	66.94	25.67	20.67
Identified	—	—	—	—	58.91	56.60	21.69	17.12

D2341 glucuronide	—	—	—	—	12.12	9.23	13.40	8.95
D9569	—	—	—	—	7.56	6.89	1.41	1.56
4-OH D2341	—	—	—	—	2.36	3.33	1.36	0.88
D9477	—	—	—	—	16.89	18.56	2.81	2.82
A1530	—	—	—	—	19.98	17.00	2.12	2.50
4-OH D3598	—	—	—	—	ND	1.58	ND	ND
D2341	—	—	—	—	ND	ND	0.59	0.42
Unidentified	—	—	—	—	7.62	6.60	2.10	1.62
Other	—	—	—	—	5.32	3.74	1.88	1.93

From McClanahan (1998)

ND, not detected; —, not applicable.

[a] See Figures 2 and 3 for structural identity of metabolites.

[b] Sum of values for 6, 12, 24, 48, 72, and 96-h urine samples (average of three rats per dose per sex combination; as a percentage of the administered dose). Sum of values for 6, 12, 24, 48, and 72 h urine sample from bile-cannulated rats (average of three animals).

[c] Sum of values for 6, 12, 24, 48, 72, and 96-h urine sample (average of three out of five animals; as a percentage of the administered dose). Sum of values for 6, 12, 24, 48, and 72-h urine sample from bile-cannulated rats (average of three animals).

[d] Samples were pooled from 0–24 h collection at 10 mg/kg bw and from 0–72 h at 1000 mg/kg/bw. 'Other' consists of diffuse, low-level radioactivity, no discernible components.

The single-dose study showed that radiolabelled bifenazate was extensively metabolized in rats given a dose at 10 mg/kg bw in contrast to rats at 1000 mg/kg bw, in which metabolism was less extensive; a large proportion of the dose was excreted in the faeces as unchanged bifenazate (61.26% and 47.88% for males and females, respectively; Table 6). The major metabolites of bifenazate in the faeces and urine resulted from hydrazine oxidation, demethylation, ring hydroxylation, cleavage of the hydrazine-carboxylic acid portion of the molecule and conjugation with glucuronic acid and sulfate (see Figure 3).

A total of eight metabolites and parent bifenazate were identified in the faeces, while three metabolites were identified in urine. The parent compound, bifenazate, was identified in the faeces of rats at both the lowest and highest doses; it represented < 7.2% of the lowest dose in male and female rats, but > 48% of the highest dose. This difference reflects the fact that a significant amount of the compound was not systemically absorbed at the highest dose. The glucuronide metabolite of bifenazate, found in relatively consistent proportions in male and female rats at both doses, arose from conjugation of the hydrazine moiety of the parent compound. This metabolite, one of the first formed, was one of the precursors to subsequent metabolic attack. The metabolite 4-OH bifenazate was formed from hydroxylation of the aromatic ring of bifenazate glucuronide and was found in slightly higher concentrations in the faeces of females than of males. Further metabolism of 4-OH bifenazate with subsequent loss of the hydrazine moiety resulted in the glucuronide or sulfate conjugate, D9477. This metabolite represented < 3% of the radioactivity in the faeces of rats at 10 mg/kg bw and < 1.2% of the radioactivity in rats at the highest dose. D9477 was further demethylated to form the metabolite D9569 which appeared as both glucuronide and sulfate conjugates.

An alternate metabolic pathway identified in the faeces led to the formation of D3598 which arose from the oxidation of the hydrazine moiety. This metabolite represented approximately 5% of the administered dose in the faeces of male and female rats at 10 mg/kg bw but < 2% of the dose in rats at 1000 mg/kg bw. Scission of the hydrazine–carboxylic acid portion resulted in the formation of D1989. With further demethylation of D1989, the metabolite A1530 was formed, which was identified as both the free metabolite and as a glucuronide or sulfate conjugate. In general, the metabolic profiles for male and female rats at 10 mg/kg bw were relatively consistent, with only small differences in metabolite formation between the sexes. The metabolic profile in rats at 1000 mg/kg bw reflected

limited absorption of parent compound, with a large percentage of the dose excreted in the faeces as unchanged bifenazate (61.3% for males and 47.9% for females).

Three primary metabolites were identified in the urine, all resulting from the loss of the hydrazine–carboxylic acid portion and demethylation of the parent compound. These were identified as glucuronide or sulfate conjugates of D9569 and the sulfate conjugate A1530. Slight differences in the concentration of metabolites in the urine were identified with male rats at the lowest dose producing more of the glucuronide conjugate D9569 than females at the lowest dose, while female rats at the lowest dose produced slightly more of the A1530 sulfate conjugate. Lower relative concentrations of these metabolites were found in the urine of male and female rats at the highest dose, which may represent saturation of the metabolic pathways leading to their formation.

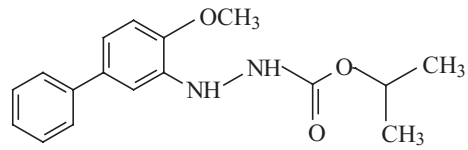
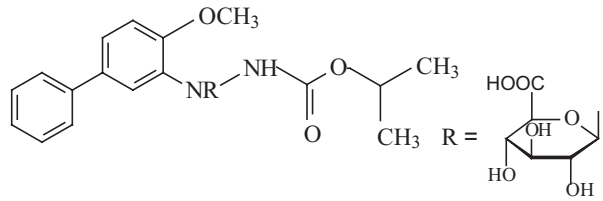
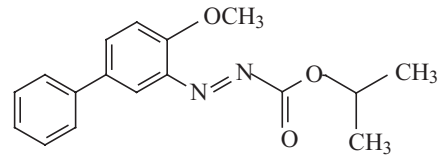
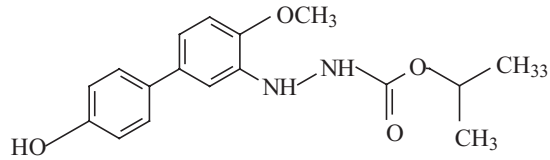
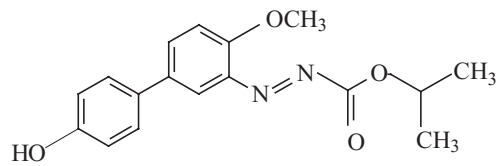
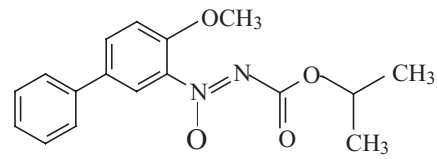
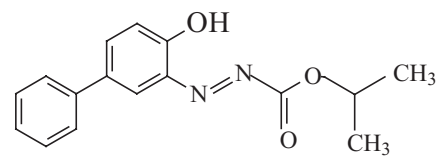
Six metabolites were identified in the bile of rats given bifenazate. These metabolites were also identified in the faeces and arose from the same metabolic pathways; however, variations in their concentrations between bile and faeces may represent further metabolism by gut bacteria. For example, the glucuronide metabolites of bifenazate, D9569, D9477, and A1530 were found in the bile of male and female rats at the lowest dose at significantly higher concentrations than in the faeces. No significant differences in the concentration of biliary metabolites in male and female rats at the lowest dose were identified. The glucuronide conjugate of parent bifenazate was the primary metabolite identified in the bile of male and female rats at the highest dose and was present in relatively the same concentrations as in rats at the lowest dose. However, the remaining metabolites were found in considerably lower concentration which suggests saturation of subsequent metabolic pathways leading to their formation (McClanahan (1998).

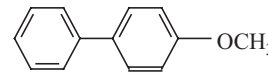
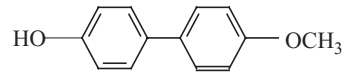
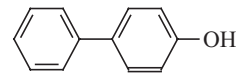
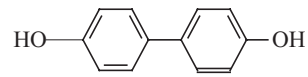
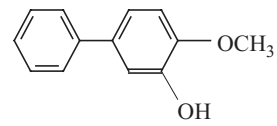
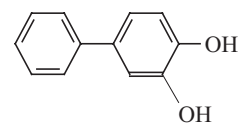
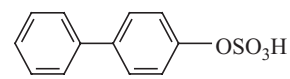
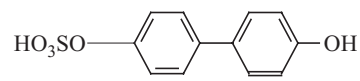
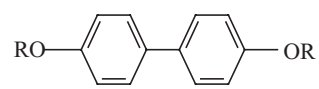
In a study of repeated doses, three major metabolites were identified, accounting for approximately 90% of the radioactivity in urine samples (22.49 and 21.27% of the administered dose identified in males and females, respectively). All components were sulfate conjugates of 4-hydroxybiphenyl or 4,4'-biphenol. In faeces, bifenazate accounted for 3.6% and 4.5% of the total radioactivity in faeces in males and females, respectively. The glucuronide conjugate of 4-hydroxybifenazate was also identified and accounted for 10.4 and 12.0% of the total radioactive residue in the faeces for males and females, respectively. The primary oxidation product of bifenazate, *N*-(4-methoxybiphenyl-3-yl)diazene-carboxylic acid isopropyl ester (D3598), was identified and accounted for 3.4% and 4.1% of the total radioactive residue in the faeces from males and females, respectively. 4-Hydroxy-D3598 was also identified in faeces, representing 13% and 13.8% of the radioactivity in the 0–24 h faeces samples for males and females, respectively. This metabolite was the only major component of the radioactivity in the 24–48 h faeces extracts. 4-Methoxybiphenyl was identified in faeces samples and accounted for 2.4% and 2.1% of the total radioactive residue in males and females, respectively. Bifenazate was also identified as the extra peak in the aqueous buffer extracts after lyophilization, accounting for 14.2% and 10.2% of the total radioactive residue in the faeces for males and females, respectively. The Meeting concluded that bifenazate had been formed as a result of hydrolysis of the glucuronide conjugate by the acidic buffer used (Banijamali, 2001).

2. Toxicological studies

Many of the studies in the toxicology data package provided were performed with bifenazate of 90–92% purity. Early lots of bifenazate contained an impurity coded F1879 or [1,1'-biphenyl]-3-sulfonic acid, 4-methoxy-2-(4-methoxy-[1,1'-biphenyl]-3-yl]hydrazide. This impurity was present at 5–7% weight depending on the lot; there were minor concentrations of several other impurities. In most dietary studies, the purity of the test article was taken into account in calculating the quantity to be administered. The purity of technical-grade bifenazate used in formulated products was stated to be approximately 96.7%.

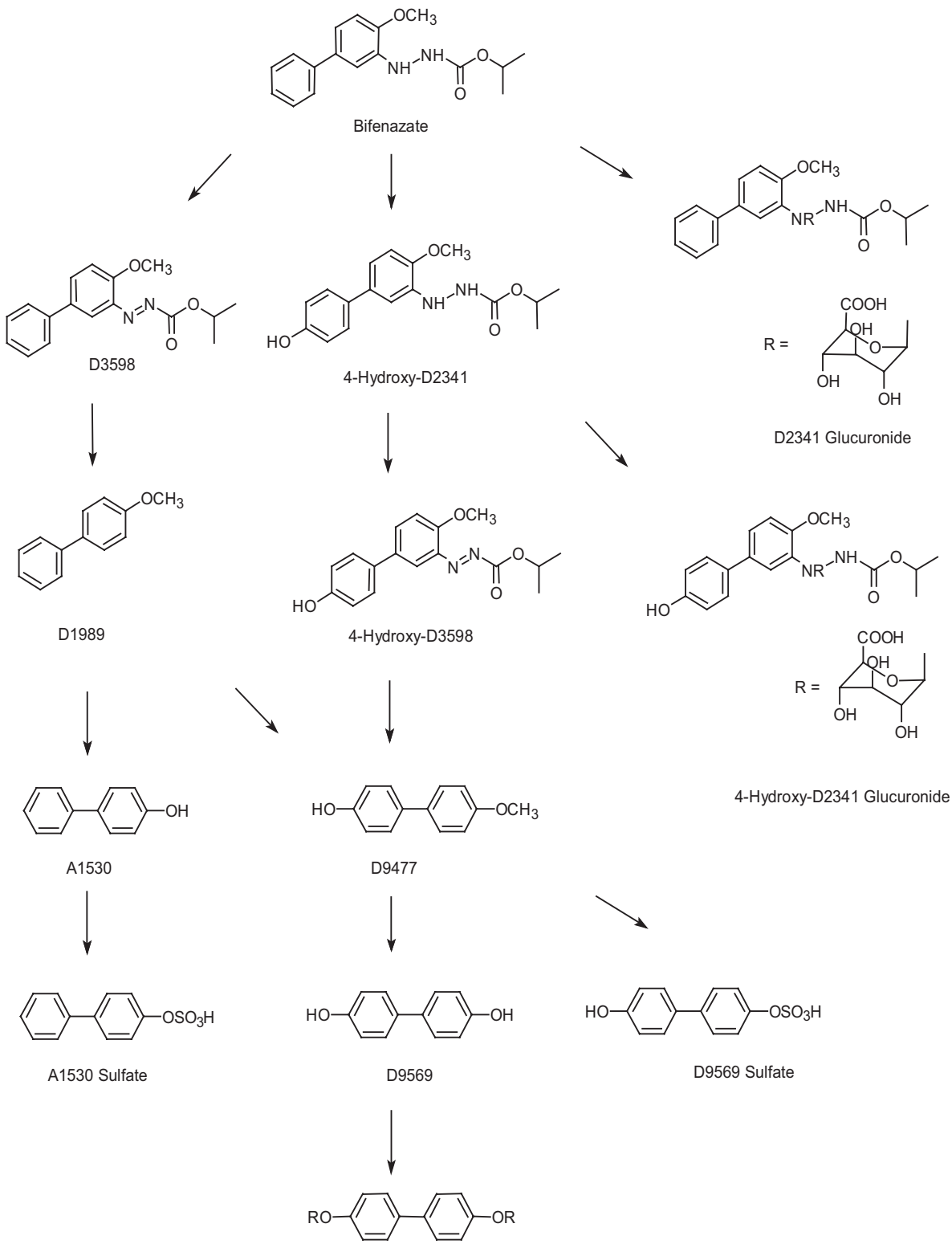
Figure 2. Chemical structures of bifenazate and its metabolites[a]

NAME/No.	STRUCTURE
Bifenazate/D2341	
D2341-Glucuronide	
Diazene/D3598	
4'-Hydroxy-D2341	
4'-Hydroxy-D3598	
D3598-N-Oxide	
Demethyl-D3598	

NAME/No.	STRUCTURE
D1989	
D9477	
A1530	
D9569	
D9963	
D9472	
A1530- Sulfate	
D9569-Monosulfate	
D9569-Sulfate or Glucuronide	

[a] The name and number assigned by the Crompton Corporation are given here.

Figure 3. Proposed metabolic pathway of bifenazate in rats



2.1 Acute toxicity

Results of studies of acute toxicity with bifenazate are summarized in Table 7.

Table 7. Results of studies of acute toxicity with bifenazate

Parameter	Species	Sex	Strain	Purity (%)	Results	Reference
Oral LD_{50}	Mice	Males and females	CD-1 [Crl: CD-1(1CR)BR]	90.4	> 5000 mg/kg bw[b]	Hoffman (1996g)[a]
Oral LD_{50}	Rat	Males and females	Sprague Dawley (Crl: CD BR)	90.4	> 5000 mg/kg bw	Hoffman (1996c)[a]
Dermal LD_{50}	Rat	Males and females	Sprague Dawley (Crl: CD BR)	90.4	> 5000 mg/kg bw	Hoffman (1996a)[a]
Inhalation LC_{50} (4 h nose only)	Rat	Males and females	Sprague Dawley (Crl: CD (SD) BR)	90.4	> 4400 mg/m^3 aerosol (4.4 mg/l)	Hoffman (1996b)[a]
Skin irritation	Rabbit	Males and females	New Zealand white (Hra: (NZW)SPF)	90.4	Minimal irritation	Hoffman (1996e)[a]
Eye irritation	Rabbit	Males and females	New Zealand white (Hra: (NZW)SPF)	90.4	Minimal irritation	Hoffman (1996f)[a]
Closed-patch skin sensitization—Buehler method	Guinea-pig	Males and females	Dunkin-Hartley Crl: (HA) BR	90.4	Not sensitizing	Hoffman (1996d)[a]
Skin sensitization—maximization method	Guinea-pig	Males and females	Dunkin-Hartley (Crj: Hartley)	91.4	Mild sensitization[c]	Ueda (1998)[a]
Skin sensitization—maximization method	Guinea-pig	Females	Dunkin-Hartley (Crj: Hartley)	97.9	Mild sensitization[d]	Rakhra & Donald (2001)[a]

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

[a] Study conducted under MAFF, OECD and United States Environmental Protection Agency (EPA) GLP standards.

[b] One out of five females died on day 8 after dosing, exhibited lacrimation, lethargy, irregular gait and laboured breathing.

[c] Eight out of twenty showed no sensitization; two out of ten had scores of 1 (scattered mild redness).

[d] Fifteen out of twenty had a score of 1 (discrete or patchy erythema); two out of twenty had a score of 2 (moderate or confluent erythema).

(a) Oral administration

Bifenazate (lot No. DS042795; purity, 90.4%) was given to groups of five male and five female young adult CD-1 by gavage (12.5% w/v in distilled water) as a single dose at 5000 mg/kg bw. Treated mice were subjected to gross necropsy at the end of a 14-day observation period. This study was conducted in accordance with GLP. One female died on day 8 after dosing. The female that died exhibited lacrimation, lethargy, irregular gait, and laboured breathing on day 7 after dosing. Decreased faecal volume was also noted for this female on days 2 through 5 and day 7 and for two other females on days 2 and 3 after dosing. There were no other mortalities, no treatment-related clinical signs of toxicity, and no necropsy findings or changes in body weight observed during the 14-day observation period. The median lethal dose (LD_{50}) was greater than 5000 mg/kg bw (Hoffman, 1996g).

Bifenazate (lot No. DS042795; purity, 90.4%) was given to groups of five male and five female young adult Sprague-Dawley rats by gavage (25% w/v in distilled water) as a single dose at 5000 mg/kg bw. Treated rats were subjected to gross necropsy at the end of a 14-day observation period. This study was conducted in accordance with GLP. There were no mortalities, no treatment-related clinical signs of toxicity, and no necropsy findings or changes in body weight observed during the 14-day observation period. The LD_{50} was greater than 5000 mg/kg bw (Hoffman, 1996c).

(b) Dermal administration

Groups of five male and five female young adult Sprague-Dawley (Crl:CD BR) rats were exposed for 24 h to bifenazate (lot No. DS042795; purity, 90.4%) at a dose of 5000 mg/kg bw moistened in 0.9% saline under an occlusive dressing. After 24 h, the occlusive dressing was removed and the treated site was wiped free of excess test material with dry gauze. Animals were observed for clinical signs and mortality for up to 14 days after dosing. This study complied with GLP. No deaths occurred. There were no treatment-related clinical signs or necropsy findings. All animals had normal body-weight gains. The dermal LD_{50} was greater than 5000 mg/kg bw (Hoffman, 1996a).

(c) Inhalation

Groups of five male and five female young adult Sprague-Dawley (Crl:CD BR) rats were exposed by head-nose only inhalation to bifenazate (lot No. DS042795; purity, 90.4%) as dust for 4 h at a nominal concentration of 5000 mg/m^3 (actual concentration, 4400 mg/m^3). Treated animals and controls were observed for 14 days and necropsied. This study complied with GLP. No mortalities occurred. No treatment-related effects were observed on body weights, except that one male and one female lost weight during the first week. Treatment-related clinical signs including moist rales, chromodacryorrhoea, and/or red/brown nasal discharge were noted in all rats after the exposure. All rats recovered by day 9. No abnormalities were noted at gross necropsy. The median lethal concentration (LC_{50}) was greater than 4400 mg/m^3 (Hoffman, 1996b).

(d) Dermal irritation

In a study of primary dermal irritation, groups of three male and three female young adult New Zealand White rabbits wereexposed for 4 h to 0.5 g of bifenazate (lot No. DS042795; purity, 90.4%) moistened with 0.5 ml of 0.9% physiological saline. Animals were then observed for 3 days. Irritation was scored by the Draize method at 30 min, 24 h, 48 h, and 72 h after removal of the dressing. This study complied with GLP. No mortalities were observed. Very slight erythema was noted in two out of six rabbits 30 min after patch removal, with resolution by 24 h. No oedema was noted on any rabbits. The primary irritation index was 0.1, i.e. bifenazate was minimally irritating to the eye under the conditions of this study (Hoffman, 1996e).

(e) Ocular irritation

In a study of primary ocular irritation, 0.1 ml of bifenazate (equivalent to 54 mg; lot No. DS042795; purity, 90.4%), was instilled into the conjunctival sac of one eye of groups of three male and three female young adult New Zealand White rabbits. Irritation was scored by the Draize method at 1 h, 24 h, 48 h, and 72 h after exposure. This study was conducted in accordance with GLP. No corneal opacity or iritis was found in the treated rabbits. All rabbits exhibited slight conjuctival irritation for up to 24 h after exposure. Bifenazate was considered minimally irritating to the eye under the conditions of this study (Hoffman, 1996f).

(f) Sensitization

In a study of dermal sensitization with bifenazate (lot No. DS042795; purity, 90.4%), 10 young male and female Dunkin Hartley guinea-pigs (Crl: (HA) BR) were tested using the Buehler method. For the induction and challenge phase, the test article was moistened with saline and applied to the skin of each animal in Hilltop chambers. For the induction phase, animals were treated dermally with the test article three times (once per week). The Hilltop chambers were left for 6 h. The challenge took place 2 weeks after the last induction. At the same time as the challenge phase, a control study of irritation was done, in which the same challenge procedure was used with naïve animals to verify that any reactions seen were not the result of irritation. The skin reactions were scored 24 h and 48 h after the induction and challenge exposures. This study complied with GLP. No deaths or abnormal signs were observed during the study. Body weights and body-weight gains were not affected by the treatment. No dermal irritation was observed at any time during the induction or the challenge phases. Bifenazate is not a skin sensitizer in guinea-pigs, as determined by the Buehler method (Hoffman, 1996d).

In a study of dermal sensitization with bifenazate (lot No. CPL 00492; purity, 91.4%), young female Dunkin-Hartley guinea pigs (Crj:Hartley) were tested using the Magnusson & Kligman maximization method. The test substance was mixed with white petrolatum. In this study, the test concentrations chosen were 2.5% for intradermal induction, 50% for topical induction, and 1% for the challenge. Skin reactions at the challenge sites were observed at 24 h and 48 h after removal of the patch. This study was conducted in accordance with GLP. Eighteen out of 20 animals in the treatment group exhibited reaction scores of 0 (no reaction) and two animals exhibited a score of 1 (scattered mild redness) to the challenge. This study indicated a mild potential for skin sensitization with bifenazate (Ueda, 1998).

In another study of dermal sensitization with bifenazate (batch No. SI 7231 lot 4; purity, 97.9%), young female Dunkin-Hartley guinea pigs (Crj:Hartley) were tested using the Magnusson & Kligman maximization method. The test substance was mixed with water. In this study, the test concentrations chosen were 6% for intradermal induction, 60% for topical induction, and 60% for the challenge. Skin reactions were observed 24 h after intradermal induction and topical application. Skin reactions were observed 24 h and 48 h after the challenge. This study was conducted in accordance with GLP. Discrete or patchy erythema was noted in all treated and control groups at 24 h after intradermal induction. No erythema was noted in any of the animals after topical application. After challenge with 60% bifenazate, positive responses were noted in 17 out of 20 animals. Discrete or patchy erythema was seen in 15 animals. Moderate or confluent erythema was noted in two animals. This study indicated a mild potential for skin sensitization with bifenazate (Rakhra & Donald, 2001).

2.2 Short-term studies of toxicity

(a) Dietary studies

Mice

In a 28-day dietary study, groups of 10 male and 10 female Crl:CD-1(ICR)BR mice (Charles River Laboratories, Raleigh, North Carolina, USA) were fed bifenazate (lot No. DS042895; purity, 91%) at a dietary concentration of 0, 200, 1000 and 5000 ppm. The consumption of compound for males was measured to be 0, 33.9 or 154.8 mg/kg bw per day at 0, 200 and 1000 ppm, while in females it was 0, 46.7 and 180 mg/kg bw per day at 0, 200 and 1000 ppm. Mice were housed individually in controlled conditions with free access to food and water. During

the study, diets were analysed for homogeneity, stability and concentration. Mice were observed twice per day for mortality and moribundity, with a more detailed observation daily and a detailed physical examination weekly. Body weight and food consumption were measured weekly. An ophthalmoscopy examination was performed before dosing and in week 4. Blood and urine were collected at the end of the study. Haematological parameters examined were erythrocyte counts, total and differential leukocyte count, haemoglobin, erythrocyte volume fraction, platelet count and cell morphology. The clinical chemistry parameters examined were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, creatinine, glucose and blood urea nitrogen (BUN). The urine was examined and the standard test parameters specified were examined. All animals were examined post mortem. The weights of the adrenals, brain (with brainstem), kidneys, liver (with gallbladder) and testes with epididymides were measured at terminal sacrifice. All tissues in the standard test parameters were preserved. Histopathological examination was done of all tissues from controls and the group at the highest dose and on all animals found dead. The lung, liver, kidney and gross lesions from all groups were examined, and where abnormalities were found in the tissues of mice at 5000 ppm, tissues from all groups were examined. The study was conducted according to United States Environmental protection Agency (EPA) test guideline 82-1, according to GLP (US EPA, Japan MAFF, and OECD).

The diet was found to be of suitable homogeneity, concentration and stability throughout the test. Mortality was seen from 1000 ppm, with 0, 0, 2, 10 and 10 males and 0, 0, 10, 10 and 10 females dying at 0, 200, 1000, 2500 and 5000 ppm, respectively. Deaths were seen up to day 8 at 2500 and 5000 ppm, and from days 13 to 22 in females and on days 24 and 29 in males at 1000 ppm. Abnormal clinical signs at 1000 ppm and above included head tilt, hunched posture, ataxia, circling, hypoactivity, tremors, limited use of front and/or hind limbs, rough hair-coat, urine stains, and dyspnoea and polypnoea in females. Decreased body-weight gain was seen from 200 ppm, with males 36% lower and females 60% lower than controls at 200 ppm. At 1000 ppm, mice lost weight during the study. Food consumption was unaffected at 200 ppm, while at 1000 ppm it was decreased by 18 or 19%. There were no treatment-related abnormalities on ophthalmoscopic examination.

On haematological examination, erythrocyte count was decreased in males at 1000 ppm, with slight decreases at 200 ppm. Leukocyte and lymphocyte counts were decreased in females at 200 ppm, with non-dose-related decreases seen in males. These findings are presented in Table 8.

Table 8. Haematological effects in mice fed diets containing bifenazate for 28 days

Parameter	Dietary concentration (ppm)				
	Males			Females	
	0	200	1000	0	200
Erythrocyte count (10^6/ml)	9.95	9.48	9.11*	9.79	9.70
Leukocyte count (10^6/ml)	4.8	4.3	4.3	4.9	3.1*
Lymphocyte count (10^6/ml)	3.4	3.1	3.2	3.9	2.2*

From Trutter (1997a)

* $p < 0.05$

On clinical chemistry examination, increased ALT activities were seen in males at 200 and 1000 ppm and in females at 200 ppm. Slightly increased alkaline activities were seen in males at 1000 ppm, while slightly increased BUN concentrations were seen in females at 200 ppm. These findings are presented in Table 9.

Table 9. Clinical chemistry effects in mice fed diets containing bifenazate for 28 days

Parameter	Dietary concentration (ppm)				
	Males			Females	
	0	200	1000	0	200
Alanine aminotransferase (U/l)	34	50*	90*	41	72*
Alkaline phosphatase (U/l)	111	124	146	154	150
Blood urea nitrogen (mg/dl)	34	28	32	20	30*

From Trutter (1997a)
* $p < 0.05$

There were no abnormalities on urine analysis. On gross examination of mice that died during the study, enlarged livers were seen in eight females and eight males at 5000 ppm, while some mice had dark lungs with mottled areas. There were no abnormalities found on gross examination of animals at the terminal sacrifice. On measurement of organ weights, the absolute weight of the testes was decreased in males at 1000 ppm; however, as the weights relative to body weight were unaffected, this was considered to be caused by body-weight loss. Absolute kidney weights were decreased in males at 1000 ppm and females at 200 ppm, with weights relative to body weight decreased in males. Absolute liver weights were increased in males at 1000 ppm, with weights relative to body weight increased at 200 and 1000 ppm. Organ weights are presented in Table 10.

Table 10. Organ weight changes in mice fed diets containing bifenazate for 28 days

Organ weight	Dietary concentration (ppm)				
	Males			Females	
	0	200	1000	0	200
Absolute kidney weight (g)	0.54	0.52	0.41*	0.39	0.35*
Kidney weight relative to body weight (%)	1.907	1.933	1.757	1.732	1.782
Absolute liver weight (g)	1.31	1.38	1.55*	1.18	1.08
Liver weight relative to body weight (%)	4.611	5.092*	6.658*	5.229	5.458

From Trutter (1997a)
* $p < 0.05$

On histopathological examination, liver findings included centrilobular necrosis, hepatocyte hypertrophy, fatty change and pigment deposition, which were seen from 1000 ppm. Congestion in the spleen was seen mainly in animals dying during the study at 1000, 2500 or 5000 ppm, while lymphoid depletion and necrosis were also observed. Increased pigment in spleen was seen in females from 200 ppm, and in males at 1000 and 2500 ppm. Lymphoid depletion in the lymph nodes (both mesenteric and mandibular) was seen from 1000 ppm in females and 2500 ppm in males. The incidences of these findings are presented in Table 11.

Table 11. Histopathological findings in mice fed diets containing bifenazate for 28 days

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	200	1000	2500	5000	0	200	1000	2500	5000
Liver										
Centrilobular necrosis	0	0	0	4	3	0	0	0	2	3

Hepatocyte hypertrophy	0	0	5	3	4	0	0	1	0	2
Fatty change	0	0	0	0	2	0	0	0	0	5
Pigment deposition	0	0	8	0	0	0	0	0	0	0
Spleen										
Congestion	0	0	3	5	7	0	0	7	7	7
Lymphoid depletion	0	0	3	7	9	0	0	9	10	10
Lymphoid necrosis	0	0	0	2	4	0	0	3	7	8
Increased pigment	0	0	8	3	0	0	5	8	1	0
Lymph nodes										
Mesenteric lymph node – lymphoid depletion	0	0	2	5	8	0	0	5	7	9
Mandibular lymph node – lymphoid depletion	0	0	0	4	8	0	0	5	6	6

From Trutter (1997a)

In this study, effects were seen in females at the lowest dose tested of 200 ppm, equal to 46.7 mg/kg bw per day (Trutter, 1997a).

In a 90-day study of oral toxicity, groups of 10 male and 10 female Crl:CD-1(ICR)BR mice were given diets containing bifenazate (lot No. DS042895; purity, 92.4%) at a concentration of 0, 50, 100, or 150 ppm (equal to 0, 8, 16.2 or 24 mg/kg bw per day in males and 0, 10.3, 21.7 or 32.9 mg/kg bw per day in females). Diets were prepared weekly and refrigerated. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice per day for signs of toxicity and mortality, with detailed cage-side observations done once daily. Body weight and food consumption were measured weekly. An ophthalmoscopic examination was done before dosing and at the end of the study. Blood was collected from all animals at terminal sacrifice for measurement of haematological and clinical parameters. Urine analysis was performed on all animals at terminal sacrifice. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed. Tissues were collected for histological examination from the control mice and mice at the highest concentration (150 ppm) and the lungs, liver, kidney, and spleen, and any gross lesions examined histopathologically. This study was conducted in accordance with GLP.

The test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. The test substance concentration analysis indicated that the measured concentrations ranged between 98.7% and 104% of the target concentrations.

No deaths occurred during the study. No compound-related clinical signs or ophthalmoscopic observations were observed in any of the test groups. Body weight, body-weight change, food consumption, and efficiency of food use showed no evidence of any compound-related changes. There were no treatment-related changes on haematological, clinical chemistry or urine analysis examination. On gross examination post mortem, two males at 150 ppm and one female at 100 ppm had pale livers. The liver weight relative to body weight was increased in males at 100 and 150 ppm and in all groups of treated females (not in a dose-related manner), while the absolute liver weight was increased in females at 150 ppm. However, as these findings were not associated with changes in liver enzyme activities or histopathological changes, they were not considered to be treatment-related. Microscopic examination of tissues showed an increased incidence and/or severity of pigment (morphologically compatible with haemosiderin) in the spleens of mice at 100 and 150 ppm (1, 1, 3, 5 males and 8, 9, 10, 10 females were affected at 0, 50, 100 and 150 ppm, respectively).

The degree of this finding was minimal in the males at 100 ppm, minimal to slight in the females at 100 ppm and males at 150 ppm, and slight to moderate in the females at 150 ppm.

The no-observed-adverse-effect level (NOAEL) was 50 ppm (equal to 8.0 mg/kg bw per day in males and 10.3 mg/kg bw per day in females). The lowest-observed-adverse-effect level (LOAEL) was 100 ppm, equal to 16.2 mg/kg bw per day in males and 21.7 mg/kg bw per day in females, for an increased incidence and severity of pigment deposition in the spleen (Trutter, 1997b).

Rats

In a 28-day dietary study, groups of 10 male and 10 female Sprague-Dawley derived [Crl CD(BR)] rats (Charles River Laboratories, Raleigh, North Carolina, USA) were housed individually in controlled conditions with free access to food and water and fed diets containing bifenazate (lot no. DS042895; purity, 91%) at a concentration of 0, 500, 1000, 5000 or 10 000 ppm. The achieved doses in males were 0, 33.3, 66.4, 319.4 and 410.4 mg/kg bw per day at 0, 500, 1000, 5000 and 10 000 ppm, respectively, and in females were 0, 35.3, 81.6 and 396.5 mg/kg bw per day at 0, 500, 1000 and 5000 ppm, respectively. The achieved dose for females at 10 000 ppm could not be calculated owing to high rates of early mortality. The diet was sampled at intervals throughout the study, and the homogeneity, stability and concentration assessed. Rats were observed twice per day for mortality and moribundity, with a more detailed cage-side observation done daily. Rats were given a detailed physical examination weekly. Body weight and food consumption were measured weekly. An ophthalmoscopic examination was done before dosing and in week 4. Blood and urine samples were collected at the end of the study. Haematological examinations included erythrocyte count, total and differential leukocyte count, erythrocyte volume fraction, haemoglobin and platelet counts. Clinical chemistry examinations included ALT, AST, alkaline phosphatase, albumin, globulin, total protein, glucose, bilirubin, creatinine, BUN, calcium, phosphorus, potassium, sodium and chloride concentrations. The urine was analysed for the standard test parameters. All animals dying during the study, and all rats killed at the terminal sacrifice were examined for gross abnormalities. At the terminal sacrifice, the weights of the adrenals, brain and brainstem, kidneys, liver and testes with epididymides were measured. All standard tissues were collected and preserved. These tissues were examined in control rats and those at the highest dose and for all animals found dead. The lung, liver, kidney, brain with brainstem, spleen, thymus, mesenteric and mandibular lymph nodes, sternal and femoral bone marrow, seminal vesicles and mandibular salivary gland from all animals were examined. The study was conducted according to US EPA test guideline 82-1, according to GLP (US EPA, Japan MAFF, and OECD).

The diet was found to be of acceptable homogeneity, stability and concentration throughout the study. Deaths were seen in males at 10 000 ppm (9 out of 10 rats), and in females at 5000 ppm (six of 10) and 10 000 ppm (10 out of 10). Deaths were seen from day 14. No abnormal clinical signs were seen at 500 or 1000 ppm, and were only seen from week 2 in females at the highest dose or week 3 in males at the intermediate and highest doses. At 5000 and 10 000 ppm, common signs included ataxia, hypersensitivity to touch, pale appearance, rough hair-coat, hunched posture and cold to the touch. Less frequent signs included dyspnoea, abnormal respiratory sounds, anorexia, prostration, head tilt, partial closure of eyes, circling and tremors. There were no ophthalmological abnormalities at the end of the study. Body-weight gain and food consumption were decreased in all treated groups.

On haematological examination, decreased erythrocyte count, erythrocyte volume fraction and haemoglobin concentration were seen in males and females at 500 ppm and above. While results were obtained from the surviving male at 10 000 ppm, these were not included in Table 12 as they are of limited significance.

Table 12. Haematological abnormalities in rats fed diets containing bifenazate for 28 days

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	500	1000	5000	0	500	1000	5000
Erythrocyte count (10^9/ml)	7.66	7.44	7.05*	6.35*	7.71	6.93*	6.34*	5.99*
Haemoglobin (g/dl)	15.4	14.4	14.6*	13.8*	16.0	14.3*	13.6*	13.2*
Erythrocyte volume fraction	0.420	0.417	0.408	0.386*	0.430	0.396*	0.372*	0.368*

From Trutter (1997c)
* $p < 0.05$

On clinical chemistry examination, BUN concentrations were increased in females at 5000 ppm. AST activities were increased in females at 5000 ppm and the male at 10 000 ppm. Alkaline phosphatase activities were decreased in all treated groups, which may be related to decreased bone alkaline phosphatase activities associated with decreased growth and lower body-weight gain. These findings are presented in Table 13.

Table 13. Clinical chemistry abnormalities in rats fed diets containing bifenazate for 28 days

Parameter	Dietary concentration (ppm)								
	Males					Females			
	0	500	1000	5000	10 000[a]	0	500	1000	5000
Blood urea nitrogen (mg/dl)	14	13	13	17	21	13	15	15	32*
Aspartate aminotrasferase (U/l)	75	79	69	90	136	71	71	66	109*
Alkaline phosphatase (U/l)	169	142*	133*	104*	106	104	77*	83	62*

From Trutter (1997c)
* $p < 0.05$
[a] There was only one survivor in this group.

There were no abnormalities detected on urine analysis. On gross examination post mortem of rats dying during the study, dark livers and dark areas in the stomach were seen in males at 10 000 ppm and females at 5000 and 10 000 ppm. At terminal sacrifice, no gross abnormalities were seen. Increased adrenal and liver weights relative to body weight were seen at 1000 ppm and above. Absolute and relative adrenal and liver weights are presented in Table 14.

Table 14. Organ-weight changes in rats fed diets containing bifenazate for 28 days

Organ weight changes	Dietary concentration (ppm)							
	Males				Females			
	0	500	1000	5000	0	500	1000	5000
Adrenal weight (g)	0.062	0.055	0.064	0.064	0.077	0.066*	0.073	0.059*
Adrenal weight relative to body weight (%)	0.015	0.014	0.020*	0.025*	0.032	0.030	0.038*	0.043*
Liver weight (g)	12.01	12.61	10.62	11.19	7.48	7.16	8.06	7.05
Liver weight relative to body weight (%)	2.91	3.13	3.24*	4.57*	3.13	3.24	4.15*	5.15*

From Trutter (1997c)
* $p < 0.05$

On histopathological examination, liver and brain changes were seen in rats at 5000 and 10 000 ppm. Splenic congestion and pigment deposition were seen in all treated rats, while lymphoid depletion was seen at 1000 ppm and above. Lymphoid depletion was seen in the mesenteric and mandibular lymph nodes and thymus at 10 000 ppm in males, and 5000 and 10 000 ppm in females. The histopathological changes are presented in detail in Table 15.

Table 15. Histopathological findings in rats fed diets containing bifenazate for 28 days

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	500	1000	5000	10 000	0	500	1000	5000	10 000
Liver										
Single-cell necrosis	0	0	0	1	10	0	0	0	3	10
Oval cell hyperplasia	0	0	0	0	9	0	0	0	0	0
Atrophy	0	0	0	0	6	0	0	0	3	4
Pigment deposition	0	0	0	1	10	0	0	0	5	8
Brain										
Vacuolization	0	0	0	6	9	0	0	0	7	8
Spleen										
Congestion	0	10	10	10	10	0	10	10	10	10
Lymphoid depletion	0	0	10	10	10	0	0	10	10	10
Increased pigment	0	10	10	10	10	0	10	10	10	10
Mesenteric lymph node										
Lymphoid depletion	0	0	0	0	9	0	0	0	7	10
Lymphoid necrosis	0	0	0	0	1	0	0	1	5	5
Mandibular lymph node										
Lymphoid depletion	0	0	0	0	8	0	0	0	0	7
Lymphoid necrosis	3	0	2	2	5	2	0	3	5	3
Thymus										
Lymphoid depletion	0	0	0	1	9	0	0	0	6	10
Lymphoid necrosis	0	0	0	1	9	2	0	2	7	8

From Trutter (1997c)

In this study, effects were seen at the lowest dietary concentration tested of 500 ppm, equal to 33.3 mg/kg bw per day in males and 35.3 mg/kg bw per day in females (Trutter, 1997c).

In a 90-day study of oral toxicity, groups of 10 male and 10 female Sprague-Dawley rats (Crl: CD BR) were given diets containing bifenazate (lot No. DS042895; purity, 92.4%) at a concentration of 0, 40, 200, or 400 ppm, equal to 0, 2.7, 13.8 or 27.7 mg/kg bw per day in males and 0, 3.2, 16.3 or 32.6 mg/kg bw per day in females. Diets were prepared weekly, refrigerated and protected from light. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for signs of toxicity and mortality, with detailed cage-side observations done once daily and a physical examination done weekly. Body weight and food consumption were measured weekly. An ophthalmoscopic examination was done before dosing and at the end of the study. Blood was collected from all animals at terminal sacrifice for measurement of haematological and clinical parameters. Urine analysis was performed on all animals at terminal sacrifice. A neurobehavioural assessment, using a functional observation battery, was done during weeks 8

and 13. The assessment used home-cage/hand-held observations and open field observations, as well as assessments of reflexes. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed. Tissues were collected for histological examination from the control rats and from those at the highest dose (400 ppm). At 40 and 200 ppm, the lungs, liver, kidney, spleen, adrenal cortex and any gross lesions were examined histopathologically. This study complied with GLP.

The test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. Measured test concentrations ranged between 102% and 105% of the target concentrations. No deaths or adverse clinical signs occurred. The neurological examination did not yield any treatment-related findings. No treatment-related ophthalmoscopic findings were observed nor were there any significant changes in urine analysis parameters. Body-weight gain was decreased by 19% and 28% in females at 200 and 400 ppm respectively, and by 26% in males at 400 ppm. Food consumption was decreased by 8% and 10% (statistically non-significant) in females at 200 and 400 ppm, respectively, and by 12% ($p < 0.05$) in males at 400 ppm. Overall food conversion efficiency was similar for all treatment groups compared to controls, except for decreased values in males at 400 ppm (16%) and females at 200 and 400 ppm (13% and 20%, respectively). Small but statistically significant and dose-related decreases in erythrocyte counts and haemoglobin values were seen in males at 400 ppm and in females at 200 and 400 ppm; these changes were considered to be toxicologically significant since they correlated with increased spleen weight and histopathological changes. Erythrocyte volume fraction values were also decreased in females at 400 ppm. Increased cholesterol was observed in all groups of treated females, and total protein was decreased in males at 200 and 400 ppm; however, these changes were considered incidental as they were of low magnitude and did not show any clear dose–response relationship. On gross examination post mortem, one male out of ten at 400 ppm had a pale kidney with a dilated pelvis. There were no other gross changes that were considered to be treatment-related. Spleen and kidney weights relative to body weight were increased in males at 400 ppm and in females at 200 and 400 ppm. Absolute liver weight was decreased in males at 400 ppm, which may be related to decreased body-weight gain; however, liver weight was increased in females at 200 and 400 ppm. Absolute and relative adrenal weights were increased in females at 400 ppm. On histopathological examination, kidney tubular cortical mineralization was seen in three of 10 females at 400 ppm, compared with one out of ten controls. A range of liver changes were seen, with centrilobular hepatocyte hypertrophy (minimal to moderate) at 200 and 400 ppm, and vacuolation, midzonal hypertrophy, extramedullary haematopoiesis and pigmented Kupffer cells (minimal) at 400 ppm. In males, individual cell necrosis (minimal to moderate) was seen at 200 and 400 ppm. In the spleen, increased pigment was seen at 200 and 400 ppm in males, with extramedullary haematopoiesis seen in females at 200 and 400 ppm and in all groups of treated males. However, at 40 ppm in males, the extramedullary haematopoiesis was only minimal, and this was not considered to be treatment-related. Vacuolation of the zona fasciculata of the adrenal cortex was seen in males at 200 and 400 ppm.

The NOAEL was 40 ppm, equal to 2.7 and 3.2 mg/kg bw per day in males and females, respectively. The LOAEL was 200 ppm, equal to 13.8 and 16.3 mg/kg bw per day in males and females, respectively, for increased centrilobullar hypertrophy, liver cell necrosis, vacuolation of the zona fasciculate of the adrenal cortex and extramedullary haematopoiesis in males and decreases in body-weight gains, decreases in food consumption and food efficiency, decreases in erythrocyte counts, decreases in haemoglobin, increases in absolute liver weights, increases in relative spleen and kidney weight, and histopathogical findings in liver in females (Trutter, 1997d).

Dogs

In a 90-day study of oral toxicity, groups of four male and four female beagle dogs were given diets containing bifenazate (lot No. PP159981B; purity, 92.4%) at a dose of 0, 40, 400, or 1000 ppm

(equal to 0, 0.9, 10.4 or 25 mg/kg bw per day in males and 0, 1.3, 10.7 or 28.2 mg/kg bw per day in females). Diets were prepared weekly and stored at room temperature away from light. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for morbidity or mortality, with clinical signs checked daily and a more through examination done weekly. A detailed physical examination was performed before commencement of treatment and at 1, 2, and 3 months. Body weight and food consumption were measured weekly. An ophthalmoscopic examination was done before dosing and at the end of the study. Blood was collected from all animals before dosing, and at 1, 2, and 3 months of dosing for measurement of haematological and clinical parameters. Urine analysis was performed on all animals before dosing, and at 1, 2, and 3 months of dosing. At the end of the study, a complete postmortem was done. The adrenals, brain, kidneys, liver, pituitary, thyroid and parathyroid, heart, spleen and testes/ovaries were weighed. The organs specified were examined microscopically. This study complied with GLP.

The test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. The test substance concentration analysis indicated that the measured concentration ranged between 98% and 100% of the target concentrations. No deaths occurred during the study. No treatment-related clinical signs, ophthalmoscopic findings, or abnormalities on the physical examination were observed. Slight decreases in body-weight gain were observed in males and females at 1000 ppm. No statistically significant changes in weekly food consumption were reported for any treatment group compared with controls. Erythrocyte count, haemoglobin and erythrocyte volume fraction values were decreased at 400 and 1000 ppm in males and females, with a compensatory increase in reticulocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), anisocytosis and platelet counts. Alkaline phosphatase and cholesterol values were increased in males at 1000 ppm after 3 months of treatment. A decrease in the percentage of protein peak 4 (not defined) on electrophoresis was noted in males and females at 1000 ppm and in females at 400 ppm. However, total protein, albumin, and globulin values were not altered. Brown coloration of the urine was seen in males at 400 ppm (one out of four animals) and at 1000 ppm (three out of four).

Increases in urinary bilirubin were also seen in males at 1000 ppm; the author suggested that this increase could be due to alteration in erythrocytic parameters. Absolute and relative liver weights were increased in males and females at 400 and 1000 ppm. No treatment-related gross pathological findings were observed in any organ. Centrilobular or diffuse hepatocellular hypertrophy was observed in females at 400 ppm and in males and females at 1000 ppm. In addition, brown pigment in Kupffer cells was seen in males and females at 400 and 1000 ppm.

The NOAEL was 40 ppm, equal to 0.9 mg/kg bw per day in males and 1.3 mg/kg bw per day in females. The LOAEL was 400 ppm, equal to 10.4 mg/kg bw per day in males and 10.7 mg/kg bw per day in females, for alterations in haematological parameters (decreases in erythrocytes, haemoglobin, and erythrocyte volume fraction, increases in reticulocytes, MCV, MCH, anisocytosis and platelets), a decrease in protein peak 4 (females only), brown-coloured urine (males only), increases in absolute and relative liver weights, hepatocellular hypertrophy (females only), and brown pigment in Kupffer cells.

The diet containing bifenazate at the lowest concentration (40 ppm) was determined to be unstable at the storage conditions used at the beginning of the study. Unfortunately, this was not discovered until week 5. Consequently, animals at the lowest dose were ingesting less compound than anticipated during the first 5 weeks. This deficiency was not insignificant, but it is noted that a NOAEL of 40 ppm was found in a 1-year study of toxicity (Goldenthal, 1997).

In a 1-year study of oral toxicity, groups of five male and five female beagle dogs were given diets containing bifenazate (lot No. PP159981B; purity, 92.4%) at a concentration of 0, 40, 400, or 1000 ppm, equal to 0, 1.01, 8.95 or 23.94 mg/kg bw per day in males and 0, 1.05, 10.42 or 29.19 mg/kg bw per day in females. Diets were prepared weekly and stored at room temperature. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were

inspected twice daily for morbidity or mortality and clinical signs. Detailed clinical examinations were performed weekly. A complete physical examination was performed on each dog before study initiation and during months 3, 6, 9, and 12. Body weight and food consumption were measured weekly. An ophthalmoscopic examination was done before dosing and at 6 and 12 months. Blood was collected from all animals before dosing, and at 3, 6, and 12 months of dosing, for measurement of haematological and clinical parameters. Urine analysis was performed on all animals before dosing, and at 3, 6, and 12 months. At the end of the study, a complete postmortem was carried out. The adrenals, brain, kidneys, liver, pituitary, thyroid and parathyroid, heart, salivary gland and testes/ovaries were weighed. The organs specified were examined microscopically. This study complied with GLP.

The test article was evenly distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. Measured test concentrations ranged between 97% and 101% of the target concentrations. No deaths occurred during the study. No treatment-related clinical signs, ophthalmoscopic findings, or abnormalities on the physical examination were observed. Body weights were comparable between treated and control animals throughout the study. Overall (weeks 0–52), body-weight gains were slightly decreased in the animals at 400 ppm (males, 14%; females, 19%) and 1000 ppm (males, 14%; females, 23%) animals. These differences were not statistically significant and reflected large, erratic changes in body-weight gains in all treated groups at various times during the study which were considered unrelated to treatment. Mean food consumption was decreased in males at 400 and 1000 ppm (17% and 12%, respectively). Decreased food consumption in males was not observed to occur in a dose-related manner and food consumption was not affected in females. Overall, food conversion efficiency values [100 × body-weight change (g) / food consumed (g)] were generally comparable, allowing for the observed erratic changes in body weight and food intake. Test article-related alterations in haematology parameters were observed at 400 and 1000 ppm at all analysis time-points (Table 16). The following increases in haematology parameters were observed at 1000 ppm at months 3, 6, and 12: (i) leukocytes in males; (ii) platelets in males and females; (iii) reticulocyte counts in females; (iv) segmented neutrophils in males; and (v) increased mean cell volume in females. The following decreases in haematology parameters were observed in the animals at the highest dose at months 3, 6, and 12: (i) erythrocytes in males and females; (ii) haemoglobin in males and females; and (iii) erythrocyte volume fraction in males and females. At 400 ppm, the following differences in haematological parameters were noted: (i) platelets were increased in males and females (months 3, 6, and 12); (ii) leukocytes were increased in males at months 3, 6 and 12; (iii) erythrocytes were decreased in males and females at months 3, 6, and 12; (iv) segmented neutrophils were increased in males (months 3, 6 and 12) and in females (months 3 and 6); (v) haemoglobin was decreased in females at month 3; (vi) mean cell volume was increased in males and females at months 3, 6, and 12; (vii) reticulocyte counts were increased in males and females at months 3, 6, and 12; and (viii) erythrocyte volume fraction was decreased in females at month 3.

Table 16. Haematological findings in beagle dogs fed diets containing bifenazate for 1 year

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	40	400	1000	0	40	400	1000
Erythrocyte count (10^6/mm^3):								
Before treatment								
3 months	5.36	5.55	5.40	5.52	5.37	5.63	5.64	5.95**
6 months	7.19	7.09	6.12*	5.33**	7.54	7.40	5.84**	5.56**
12 months	6.79	6.96	6.23	5.46*	6.47	6.89	5.76	5.43**
	7.35	7.43	6.38	5.50**	6.46	7.00	5.96	5.04**

Haemoglobin (g/dl):								
Before treatment	13.0	13.0	12.9	12.6	13.8	13.7	14.5	14.2
3 months	16.7	16.3	15.5	13.3**	16.7	17.2	15.2*	14.0**
6 months	16.0	16.5	15.5	13.4**	15.6	16.6	14.7	13.4*
12 months	17.5	18.1	16.4	13.7**	16.2	17.6	15.7	13.0**
Erythrocyte volume fraction:								
Before treatment	0.356	0.375	0.364	0.353	0.368	0.377	0.389	0.395
3 months	0.485	0.480	0.450	0.385**	0.488	0.493	0.432**	0.406**
6 months	0.473	0.491	0.460	0.399*	0.452	0.481	0.434	0.401
12 months	0.518	0.529	0.474	0.402**	0.463	0.495	0.449	0.378**
MCV (μm^3)								
Before treatment	66.6	67.7	67.5	64.3	68.6	67.0	69.1	66.4
3 months	67.5	67.8	73.4*	72.3	64.8	66.7	74.1**	73.0**
6 months	69.7	70.5	74.0*	73.5	69.9	69.8	75.3**	74.0*
12 months	70.5	71.3	74.4	73.3	71.7	70.6	75.5*	75.0*
Platelets ($10^3/mm^3$):								
Before treatment	316	312	303	345	293	337	296	290
3 months	192	231	341*	430**	243	258	394**	399**
6 months	232	226	340	422**	259	273	429**	414**
12 months	204	191	314*	409**	249	297	392*	418**
Reticulocytes (per 100 erythrocytes):								
Before treatment	0.9	0.8	0.9	0.5	0.5	1.1	0.6	1.0
3 months	1.5	1.9	4.2*	4.0*	1.0	1.7	2.9	3.7*
6 months	0.5	0.5	2.5	3.0	0.3	0.5	2.1*	3.6**
12 months	0.4	0.1	1.4	2.2**	0.3	0.4	1.7	3.1**
Leukocyte count ($10^3/mm^3$):								
Before treatment	9.5	10.6	9.5	9.0	8.6	10.2	8.2	8.8
3 months	9.2	11.9	14.1**	15.1**	9.3	11.0	11.4	13.7*
6 months	9.6	12.4	16.1*	15.9*	10.1	11.6	12.0	13.2
12 months	10.1	11.2	14.0	15.9*	10.6	10.2	9.6	14.7
Neutrophil count ($10^3/mm^3$):								
Before treatment	635	6.8	5.3	5.7	4.8	6.8	4.4	5.2
3 months	5.8	7.8	8.7*	8.9*	5.3	6.1	7.0	8.3
6 months	6.1	8.1	11.5*	10.6*	6.7	7.4	8.1	8.4
12 months	6.9	8.1	10.1	12.1*	7.7	7.3	6.4	9.5
Lymphocyte count ($10^3/mm^3$):								
Before treatment	2.8	3.6	4.1	3.2	3.7	3.2	3.7	3.5
3 months	3.2	3.7	4.8*	5.9*	3.6	4.4	4.3	4.9
6 months	3.0	4.0	3.9	4.8	3.2	3.7	3.6	4.2
12 months	2.6	2.5	3.0	3.1	2.5	2.3	2.9	4.3*

From Goldenthal (1999)
MCV, mean corpuscular volume.
* $p < 0.05$; ** $p < 0.01$

Relative percentages of protein electrophoresis peak 4 were decreased at months 3, 6, and 12 in males at 400 ppm and 1000 ppm. It was stated that electrophoresis peak 4 corresponded to beta-globulins, "many of which function in the transport of haem or Fe and a decrease in these values may reflect increased utilisation of haem or iron, secondary to a haemolytic process." Total bilirubin

was increased in females at months 3, 6, and 12 at 400 and 1000 ppm and at some intervals in males at 400 and 1000 ppm. Other changes in clinical parameters were minor, not dose-dependent, and/or not sustained over time and considered not to be treatment-related. Increased urinary bilirubin and brown coloration of the urine were noted at 400 and 1000 ppm in males only. No treatment-related gross pathological findings were observed. Statistically significant increases in relative liver weights were observed in females at 1000 ppm. Absolute and relative weights of the adrenals and pituitary were slightly decreased in males at 1000 ppm and slightly increased in females at 1000 ppm. These were considered to be incidental findings. Kidney weights (both absolute and relative to body weight) were increased (statistically non-significant) in males and females at 400 and 1000 ppm. Test article-related microscopic pathology changes were noted in the bone marrow, kidney, and liver at 400 and 1000 ppm. Mild to moderate hyperplasia of the bone marrow of the rib, femur and sternum of the males and females was noted at 400 and 1000 ppm. This hyperplasia was characterized by a reduction in the amount of adipose tissue present in the marrow cavity, with an apparent increase in the numbers of cells of both the erythroid and myeloid series. Brown pigment was present within the tubular epithelial cells of the kidney in trace to mild amounts in males and females at 400 and 1000 ppm. The pigment occurred as discrete granules within the cytoplasm and was morphologically consistent in appearance with haemosiderin. Brown pigment was present in the liver, primarily within the Kupffer cells lining the hepatic sinusoids, in trace amounts in males and females in groups at 400 and 1000 ppm. As with the pigment in the kidney, it generally occurred as discrete granules within the cytoplasm and was morphologically consistent in appearance with haemosiderin. The changes in the haematology parameters indicate blood loss with regeneration and correspond to the increased haemosiderin pigment in the liver and kidney and hyperplasia in the bone marrow observed in the microscopic evaluation of these organs. The presence of haemosiderin, the regenerative response, and the presence of bilirubinaemia and bilirubinuria are consistent with a haemolytic mechanism.

The NOAEL was 40 ppm, equal to 1.01 mg/kg bw per day in males and 1.05 mg/kg bw per day in females. The LOAEL was 400 ppm, equal to 8.9 mg/kg bw per day in males and 10.4 mg/kg bw per day in females for changes in haematology (decreases in erythrocyte counts, haemoglobin and erythrocyte volume fraction values; increases in reticulocyte counts and MCV), clinical chemistry and urine analysis parameters (increases in serum total bilirubin, urinary bilirubin and brown coloration of the urine), and histopathological changes (mild to moderate hyperplasia of the bone marrow of the rib, femur and sternum and brown pigment in liver and kidney) (Goldenthal, 1999).

(b) Dermal administration

Rats

In a 21-day study of dermal toxicity with repeated doses, groups of 10 male and 10 female Sprague-Dawley rats (Crl:CD(BR)VAX/Plus) received dermal applications of bifenazate (lot No. PP159981B; purity, 92.5%) at a dose of 0, 80, 400 or 1000 mg/kg bw per day, 6 h per day for a total of 21 to 22 days. The test substance was formulated in distilled water. The hair was clipped from the back of each rat before the first application, then periodically as required. This clipped area comprised not less than 15% of the total body surface area. The test article was applied uniformly to the dose site by gentle inunction using a glass stirring rod. The application site was then wrapped in a gauze bandage and secured with tape for 6 h. At the end of exposure, the gauze and tape were removed and the application site rinsed with tap water and dried. Animals were observed twice daily for signs of mortality, morbidity, toxicity, and the presence of dermal irritation. A detailed clinical examination was done weekly, including observations of general condition, skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs, feet, respiration and palpation for tissue masses. Dermal reactions at the application site were scored daily (before dosing) using the Draize method. Body weight was measured before dosing, then weekly. Food consumption was measured

weekly. Blood and urine samples were taken at the end of the study, and the standard test parameters were examined. An ophthalmoscopic examination was done before dosing and at termination. At the end of the study, a postmortem examination was done on all animals. The adrenals, kidneys, liver, spleen and testes were weighed, and a microscopic examination conducted on the adrenals, kidneys, liver, lung, skin (treated and untreated) and spleen. Where no significant abnormalities were seen at 1000 mg/kg bw per day, tissues from rats at 80 and 400 mg/kg bw per day were not examined. The spleen was determined to be a target organ and was microscopically examined for the groups at 80 and 400 mg/kg bw per day. This study complied with GLP.

There were no treatment-related effects in clinical signs of toxicity, mortality, ophthalmoscopic evaluations and gross histopathological findings at necropsy. Dermal irritation (erythema/oedema) occurred more frequently in some of the treated rats of both sexes in comparison to controls, but without any dose–response relationship. The irritation was minimal and only lasted for short durations. Body weight was statistically significantly decreased in males at the highest dose (11%) and females (10%) and females at the intermediate dose (9%). Food consumption was decreased at 400 and 1000 mg/kg bw per day. In males at the highest dose, haemoglobin was decreased (5%) and platelets were increased significantly (13%). In females at the highest dose, significant decreases were seen in erythrocytes (12%), haemoglobin (10%) and erythrocyte volume fraction (9%). Slight decreases in haematological findings showed a trend toward anaemia, which was compensated by extramedullary haematopoiesis in the spleen. Mild erythrocyte hypochromasia and anisocytosis were noted in two out of ten females in the groups at 400 and 1000 mg/kg bw per day. Mild hypochromasia was also noted in three out of ten females at 1000 mg/kg bw per day. Total bilirubin was statistically significantly increased in femalea at 1000 mg/kg bw per day. Creatine phosphokinase activities were decreased in males at 400 and 1000 mg/kg bw per day, by 35% and 46% respectively, which was considered to be treatment-related. A mild increase in urinary ketones, protein, and specific gravity and a decrease in urinary volumes were seen at 400 and 1000 mg/kg bw per day. A mild increase in ketonuria was likely due to the decreased body weight of the animals. Proteinuria was not associated with any renal tubular dysfunction on the basis of histopathological examination. Absolute testes weights were unaffected by treatment; the increased relative testes weights were a result of decreased body-weight gain. Absolute and relative adrenal and spleen weights were increased in males at 1000 mg/kg bw per day, while absolute and relative spleen weights were increased in females at 1000 mg/kg bw per day. Extramedullary haematopoiesis of the spleen was seen at 400 and 1000 mg/kg bw per day. No other treatment-related histopathological changes were observed. No treatment-related effects were seen at 80 mg/kg bw per day.

The NOAEL was 80 mg/kg bw per day on the basis of decrease in body-weight gains, decrease in food consumption, clinical haematology (decreased haemoglobin, erythrocyte volume fraction and erythrocyte counts), urine analysis (increase in ketones, protein and specific gravity and decrease in urinary volume) and histopathology (extramedullary haematopoiesis) observed at 400 mg/kg bw per day, the LOAEL, and above (Goldenthal, 1998).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of oncogenicity, groups of 50 male and 50 female Crl:CD-1(ICR)BR mice were given diets containing bifenazate (lot/batch No. PP159945; purity, 90.2%) at a concentration of 0, 10, 100, or 225 ppm (males) and 175 ppm (females), equal to 0, 1.5, 15.4 and 35.7 mg/kg bw per day in males and 0, 1.9, 19.7 and 35.7 mg/kg bw per day in females, for up to 79 weeks. Diets were prepared weekly. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for mortality and morbidity. Changes in clinical condition or

behaviour were recorded daily. Detailed clinical observations were recorded weekly. Body weight and food consumption were measured weekly for the first 13 weeks, then monthly for the rest of the study. Water consumption was not measured. An ophthalmoscopic examination was not done. Blood was collected from all surviving animals in weeks 52 and 78, and the cell morphology, erythrocyte count and total and differential leukocyte counts were determined. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed. Tissues were collected for histological examination from the control group and from animals at the highest dose, animals that died prematurely, and animals sacrificed in extremis. All gross lesions and masses from all animals and the lungs, liver, and kidneys from mice at 10 and 100 ppm were examined microscopically. This study complied with GLP.

The test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. The measured test concentrations ranged between 89.6% and 110% of the target concentrations.

Survival was not significantly affected by treatment, with 80%, 84%, 94% and 90% of males and 82%, 74%, 90% and 78% of females surviving at 0, 10, 100 and 225/175 ppm, respectively. The only clinical sign which was associated with treatment was a swollen abdomen, seen in 5, 1, 6 and 12 males and 5, 3, 9 and 9 females at 0, 10, 100 and 225/175 ppm respectively. Body weights were consistently decreased in females at 175 ppm. Small but statistically significant decreases in body weight were also observed in males at 225 ppm. Body-weight gains (overall) were decreased in females (16%) at 175 ppm. Overall body-weight gains were comparable to those of controls in all males and females at 10 and 100 ppm. No treatment-related differences in food consumption or use were observed in any treated group. Erythrocyte count was decreased in males at 225 ppm, and slightly decreased in females at 175 ppm. Leukocyte and lymphocyte counts were decreased in males at 100 and 225 ppm. While all treated females had a decreased leukocyte count at 78 weeks, this would appear to be an artefact related to the increased value in controls. Absolute and relative liver weights were increased in males at the highest dose and relative weights in females at the highest dose. A decrease in kidney weights (absolute and relative) was seen in males at the intermediate and highest doses and decreased absolute kidney weights in females at the highest dose. In the absence of corroborating histopathology, the decreases in kidney weights were considered to be of equivocal toxicological importance. An increased incidence (%) of liver masses was detected in males at the highest dose (16% treated vs 6% controls). It was stated that these masses generally correlated to hepatocellular adenomas. No other treatment-related gross pathological changes were noted in any treated group. An increased incidence of primary, benign hepatocellular adenomas was observed in males at the highest dose (21% in treated groups vs 10% in controls). This incidence was greater than historical control incidences (4.3–14.9%); however, it was not statistically significant and hepatocellular carcinomas were not identified in these animals. In addition, benign hepatocellular adenoma and hepatocellular carcinoma had low occurrences in the females at the highest dose (2% each vs 0% in controls) and were within historical control ranges (benign adenoma, 0–2%; carcinoma, 0–2%).

The NOAEL was 10 ppm (equal to 1.5 mg/kg bw per day) in males and 100 ppm (equal to 19.7 mg/kg bw per day) in females. The LOAEL was 100 ppm (equal to 15.4 mg/kg bw per day) in the males (for changes in haematology parameters and possibly kidney weights) and 175 ppm (equivalent to 35.7 mg/kg bw per day) in the females (for decreased body weights and body-weight gains) (Ivett, 1999a).

Rats

In a long-term combined study of toxicity/oncogenicity, groups of 50 male and 50 female Sprague-Dawley (Crl:CD BR) rats were given diets containing bifenazate (lot/batch No. PP159945; purity, 90.2–92.5%) at a nominal concentration of 0, 20, 80, or 200 ppm (males) and 160 ppm

(females) (equal to 0, 1.0, 3.9, or 9.7 mg/kg bw per day in males and 0, 1.2, 4.8, and 9.7 mg/kg bw per day in females) for up to 104 weeks. An additional 10 rats of each sex per dose were sacrificed at 53 weeks. Diets were prepared weekly. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for mortality and morbidity. Changes in clinical condition and behaviour were recorded daily. Detailed clinical observations were recorded weekly. Body weight and food consumption were measured weekly for the first 13 weeks, then monthly for the rest of the study. Water consumption was not measured. An ophthalmoscopic examination was performed before treatment and during week 104. Blood and urine were collected from 10 rats of each sex per dose in weeks 13, 26, 52, and week 104. Haematological parameters examined were erythrocyte count, leukocyte count, erythrocyte volume fraction, haemoglobin and platelet count. Differential leukocyte count and cell morphology were assessed on the control rats and on those at the highest dose, although slides were prepared for all groups. Standard clinical chemistry and urine analysis parameters were examined. All rats dying during the study were examined grossly, and all tissues were prepared and examined histopathologically. After 52 weeks, 10 rats of each sex per group were killed and examined, and all rats were killed at the end of the study. After scheduled sacrifice, a postmortem examination was done. The adrenals, brain, kidneys, liver, ovaries, spleen and testes with epididymides were removed and examined. All tissues were examined from control rats and rats at the highest dose. At 20 and 80 ppm, the lungs, liver, spleen and kidneys, as well as any gross lesions from all rats were examined. This study complied with GLP.

The test article was uniformly distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. The measured test concentrations ranged between 90.8 to 105% of the target concentrations.

Survival was not significantly affected by treatment in either males or females. At termination (104 weeks), survival rates ranged from 40% to 78% in males and from 28% to 44% in females. No treatment-related clinical signs were observed. Slight decreases in body weights and body-weight gains were observed in females at 80 and 160 ppm and in males at 200 ppm, particularly early in the study. The body weights for females at the intermediate dose (80 ppm) and males at the highest dose (200 ppm) subsequently recovered to similar mean concurrent values. The mean body weights of females at the highest dose generally remained slightly lower than the concurrent controls for the remainder of the study. The mean overall food consumption in the animals at the highest dose was decreased but this was not statistically significant. Overall food conversion efficiency was reduced in females at the highest dose (statistically non-significant).

At terminal sacrifice, lens opacity (unilateral/bilateral/focal/multifocal) was observed in males at the intermediate and highest doses (11 and 21% respectively vs 0% in controls) and in females at the intermediate dose (14% treated vs 0% controls); the majority of the lesions occurred unilaterally. Bilateral diffuse lens opacity was not observed in any of the animals. The submitted ophthalmology report stated that the ophthalmoscopic findings may have been confounded by trauma associated with blood collection from the orbital venous plexus (of the first 10–20 rats of each sex per dose) during the study and by the higher survival rate in males at the intermediate and highest doses compared to controls (n = 35 and 39 rats, respectively, vs 27 controls). The sponsor concluded that the relationship of the observed ophthalmoscopic findings to treatment with bifenazate was uncertain.

In females at the highest dose at weeks 13, 26, and 52, decreased erythrocyte counts (6–10%), haemoglobin (5–8%), and erythrocyte volume fraction (5–7%) were detected. These parameters were also decreased in females at the highest dose at weeks 78 and/or 105 (6–9%; not statistically significant). In addition, a decrease in erythrocyte count (7%) was detected in females at the intermediate dose at week 26. The values for these haematological parameters were within historical control values. No treatment-related differences from concurrent controls

were observed in the blood smears obtained from the animals at the highest dose at weeks 13, 26, 52, 78, and 104 or in any of the moribund animals at time of sacrifice. No treatment-related differences from controls were observed in any clinical chemistry parameter. Decreases in total cholesterol were observed at weeks 26, 52, and 78 in males at the highest dose. However, no corroborating gross or histological data were observed.

There were no treatment-related abnormalities on urine analysis. There were no treatment-related macroscopic findings. Spleen weight was slightly increased in both sexes receiving the highest dose at the interim sacrifice, and in females at 160 ppm at the terminal sacrifice. Liver weight was slightly increased in males at 200 ppm at interim and terminal sacrifice and in females at 160 ppm at the terminal sacrifice. None of the organ-weight changes achieved statistical significance. At the interim sacrifice, pigment that was morphologically comparable with haemosiderin was seen as a treatment-related histomorphological finding in the spleen sections. Although the pigmentation was similar between treated and untreated animals, the severity of the lesion increased slightly in the groups of animals at 80 ppm and at the highest dose, with females exhibiting slightly more severe lesions than males. The incidence and/or severity of the spleen pigment, as seen in the interim-sacrifice animals, were not increased in the animals sacrificed at week 105 or animals dying during the study. No treatment-related neoplastic changes were observed.

The NOAEL was 80 ppm, equal to 3.9 mg/kg bw per day in males and 4.8 mg/kg bw per day in females. The LOAEL was 200 ppm for males and 160 ppm for females (equal to 9.7 mg/kg bw per day in males and females) for decreases in body weight, body-weight gain, food consumption and haematological parameters. The study author concluded that the NOEL was 20 ppm (equal to 3.9 mg/kg bw per day in males and 4.8 mg/kg bw per day in females). The difference in the NOAEL value between the study author and this reviewer is due to the difference between NOEL andNOAEL. The Meeting considered that the effects seen at 80 ppm (i.e. transient slight decreases in body weight and body-weight gain early in the study and a transient slight decrease in erythrocyte counts) were not adverse (Ivett, 1999b).

2.4 Genotoxicity

Results of studies of genotoxicity with bifenazate are shown in Table 17.

Table 17. Results of studies of genotoxicity with bifenazate

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation (Ames test)	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* strain WP2 *uvrA*	10–5000 µg/plate ± S9 in DMSO	90.2	Negative[a,b]	Wagner & Coffman (1996)
Forward mutation	L5178Y/*Tk*$^{+/-}$ mouse lymphoma cells	Initial assay: 100–5000 µg/ml − S9 25–2500 µg/ml + S9 in DMSO Independent repeat assay: 5–50 µg/ml − S9 25–500 µg/ml + S9 in DMSO	90.2	Negative[c,d,e]	San & Clarke (1996)

Cytogenetic test	Chinese hamster ovary cells (CHO-K1 cells)	Initial assay: 12–375 µg/ml −S9 20–1250 µg/ml + S9 in DMSO Independent repeat assay:[f] 12–94 µg/ml −S9 20–236 µg/ml + S9 in DMSO	90.2	Negative	Gudi & Schadly (1996)
In vivo					
Micronucleus formation[g]	Mouse (ICR)	Males: 96, 192, 384 mg/kg bw Females: 50, 100, 200 mg/kg bw	90.2	Negative[h]	Gudi (1996)

DMSO, dimethyl sulfoxide; S9, exogenous metabolic activation system from 9000 × *g* fraction of rat liver induced with Aroclor 1254.

[a] Tested in triplicate, positive controls included, statements of compliance with good laboratory practice and quality assurance included.

[b] Precipitate was observed at concentrations greater than 1000 µg per plate, but no appreciable toxicity was observed.

[c] Tested in duplicate, positive controls included, statements of compliance with good laboratory practice and quality assurance included.

[d] Initial assay failed owing to excess toxicity and high mutant frequencies among S9-activated solvent controls. The assay was repeated at 10–100 µg/ml activation −S9 and 25–1000 µg/ml +S9. The non-activated portion failed owing to excess toxicity and was repeated at 5–50 µg/ml.

[e] In the independent repeat assay, visible precipitate present at concentrations ≥ 100 µg/ml with S9 in treatment medium. Toxicity in cultures was observed at doses ≥ 20 µg/ml −S9 and ≥ 100 µg/ml +S9.

[f] In the repeat assay, cells were exposed for 20 h −S9 and 6 h +S9.

[g] Five of each sex per dose in the range-finding study; 15 of each sex per dose in the definitive study, with 20 of each sex in the group at the highest dose. Bone marrow collected at 24, 48 and 72 h. Administered via intraperitoneal injection in corn oil. Positive controls included (cyclophosphamide, 60 mg/kg bw; five males, five females). Statements of compliance with good laboratory practice and quality assurance were included.

[h] Mortality observed in 3 out of 20 males at 384 mg/kg bw. Clinical signs after dosing included lethargy at the intermediate and highest doses.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study of reproduction toxicity, groups of 30 male and 30 female Crl:CD(SD) BR rats were given diets containing bifenazate (lot/batch No. PP15 9981-B; purity, 92.5%) at a dose of 0, 20, 80, or 200 ppm, equal to 0/0, 1.5/1.7, 6.1/6.9 and 15.3/17.2 mg/kg bw per day in the parental animals and 0/0, 1.7/1.9, 6.9/7.8, and 17.4/19.4 mg/kg bw per day in F_1 animals (males/emales, respectively)]. Exposure of parental animals began at age approximately 6 weeks and lasted for 10 weeks before mating. F_1 pups (30 of each sex per dose) selected to produce the F_2 generation were exposed to the same doses as their parents, beginning on postnatal day 22. F_1 animals were dosed with the test article for 10 weeks before mating to produce the F_2 litters. Mating to produce a second F_{2b} generation was not performed.

Diets were prepared weekly and stored at room temperature. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for mortality and morbidity and detailed examinations were performed weekly. Females were weighed weekly before mating and on days 0, 4, 7, 11, 14 and 20 of gestation, and days 1, 4, 7, 14 and 21 of lactation. Food consumption was measured weekly for both sexes during the period before mating and daily during

gestation and lactation for the females; food consumption for males was recorded on a weekly basis after mating and until scheduled necropsy.

Estrous cycles were monitored with vaginal smears for 3 weeks before mating and until mating was confirmed or the mating period was completed. Semen from each adult male was evaluated for sperm concentration and motility; sperm morphology was examined in control and adult males at 200 ppm. Duration of gestation was calculated using the date delivery began. Females were allowed to deliver normally and rear young to weaning on day 21. Litters were examined after delivery and pups were sexed, examined for gross abnormalities and the number of stillborn and live pups recorded. Litters were then examined twice per day for survival. Pups dying on days 0 to 4 were examined, and pups with external abnormalities suggestive of skeletal changes were cleared and stained for skeletal examination. A detailed postmortem was done on all pups dying between day 4 and weaning, with all gross abnormalities preserved for histopathological examination. On postnatal day 4, all litters were reduced to eight pups per litter, with four pups of each sex where possible. A detailed physical examination of all pups was done on days 1, 4, 7, 14 and 21, with pups sexed and weighed at this time.

At weaning of the F_1 pups, 30 pups of each sex per dose were selected to become F_1 parents. These pups were offered treated diet from day 22. F_1 pups were examined to determine the time of balano-preputial separation (males from day 40) or vaginal perforation (females from day 30). The F_0 and F_1 adults were killed after either selection of F_1 generation or weaning of F_2 pups respectively. A complete postmortem was done. The weights of the adrenals, brain, epididymides, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, thymus and uterus were measured. A histopathological examination was done on rats at 0 and 200 ppm, and on all rats dying during the study. The adrenals, brain, epididymis, cervix, coagulating gland, kidney, liver, ovaries, pituitary, prostate, seminal vesicle, spleen, testes, thymus, uterus, vagina, vas deferens and any gross lesions present were examined. The non-selected F_1 weanlings and all F_2 weanlings were killed on postnatal day 21, with terminal investigations focusing on developmental landmarks (including balano-preputial separation, vaginal perforation, and spermatogenic end-points). The brain, spleen and thymus were collected and weighed.

The test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. Measured test concentrations in the diet ranged between 96.2% and 101% of the target concentrations.

There were no treatment-related clinical signs or mortalities observed in the parental or F_1 animals. One F_0 male at 20 ppm died during study week 8, but no deaths were observed at the higher doses, and therefore, the death at the lowest dose was not considered to be treatment-related. One F_1 female at 200 ppm was found dead during study week 37; necropsy findings suggested that this female had kidney disease as evidenced by white areas on the kidneys, dilated renal pelvis, and distended ureter, and this death was considered not to be attributed to treatment. Reduced mean body-weight gains occurred at 200 ppm in males during weeks 2–5 and in females during weeks 0–3, but body-weight gains were not significantly affected in either sex for the remainder of the 10-week period before breeding.

Mean body weights of animals at 200 ppm were lower than controls from weeks 3–19 (males) and week 1 through gestation and lactation (females). Mean body weights and body-weight gains of F_0 animals at 20 and 80 ppm were largely unaffected by test article administration through the study. In the F_1 animals at 20 and 80 ppm, decreased body-weight gains were observed throughout the interval before mating in both sexes and in females during gestation and lactation. Sporadic reductions in body-weight gains were observed in the F_1 females at 20 ppm during the period before mating. In order to assess the equivocal body-weight gain effect at this dose, a supplementary two-generation reproduction study (WIL-155040), included with the current submission, was conducted to investigate the parental body-weight reductions observed at 20 ppm. In this supplemental study,

groups of 30 males and 30 female rats were fed bifenazate at a concentration of 7.5, 15, or 20 ppm, equal to 0, 0.6, 1.1, or 1.5 mg/kg bw per day for males and 0, 0.6, 1.2, or 1.7 mg/kg bw per day for females. Mean body weights and body-weight gains were unaffected by treatment, and therefore, the decreased body-weight gains observed in the current study at 20 ppm were considered not to be treatment-related. Food consumption was not affected in F_0 rats, but was decreased intermittently in F_1 males at 80 and 200 ppm and F_1 females at 200 ppm in the period before mating.

There were no treatment-related effects on fertility or gestation length in either generation, nor were there any treatment-related effects on sperm numbers, production, motility or abnormalities. There were no treatment-related effects on the mean litter size in either generation. In both generations, there was a slight decrease in the percentage of males at 200 ppm, but this was within the range for historical control values and was not considered to be treatment-related. There were no treatment-related effects on survival during lactation, and no developmental effects detected in pups dying during the first 4 days. No treatment-related effects on pup body weight were seen during lactation, and no effects were seen on postmortem examination of surplus pups in the F_1 generation or of weanling F_2 pups. There were no effects on balano-preputial separation; however, vaginal perforation was delayed in female pups at 80 and 200 ppm, occurring at 36, 34, 40 and 47 days at 0, 20, 80 and 200 ppm. There were no treatment-related gross pathological findings in the F_0 or F_1 adults. No treatment-related changes in organ weights were observed in the F_0 or F_1 adults. A decrease in absolute liver weight was seen in F_1 females at 200 ppm, which paralleled a slightly lower body weight and was not considered to be toxicologically significant. The spleen weight (absolute and relative) was increased in F_0 females at 200 ppm, with the relative spleen weight increased in F_1 females at 200 ppm. Increases were also seen in relative adrenal and kidney weights in females at 200 ppm; however, these were not considered to be treatment-related, but to be related to body-weight changes. No treatment-related microscopic changes were observed in the F_0 or F_1 animals.

The NOAEL for parental toxicity was 20 ppm, equal to 1.5 and 1.7 mg/kg bw per day in males and females, respectively. The LOAEL for parental toxicity was 80 ppm, equal to 6.1 and 6.9 mg/kg bw per day in males and females, respectively, on the basis of decreased body weights and body-weight gains. The NOAEL for reproductive and neonatal toxicity was 200 ppm, equal to 15.3 and 17.2 mg/kg bw per day in males and females, respectively, the highest dose tested (Schardein, 1996).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 25 pregnant Sprague-Dawley (Crl:CD BR) rats were given bifenazate (lot No. PP159981-B; purity, 92.5%) at a dose of 0, 10, 100 or 500 mg/kg bw per day (not adjusted for purity) in 0.5% carboxymethyl cellulose by gavage on days 6–15 of gestation, inclusive. Stability, homogeneity and dose concentrations were confirmed analytically. All animals were observed twice daily for mortality and moribundity and once per day for clinical signs of toxicity. Animals were also observed for signs of toxicity approximately 1 h after dosing. Maternal body weights and food consumption were recorded on day 0 of gestation, daily on days 6–16 of gestation, and on day 20. On day 20 of gestation, all surviving dams were sacrificed and subjected to gross necropsy. The uterus and ovaries were exposed and the number of corpora lutea on each ovary was recorded. Gravid uteri were weighed, opened, and the location and number of viable and non-viable fetuses, early and late resorptions, and the total number of implantations were recorded. Uteri from females that appeared non-gravid were opened and placed in 10% ammonium sulfide to detect any early implantation loss. Maternal tissues were preserved for histological examination only as indicated by gross findings. All fetuses were weighed, sexed, and examined for external malformations/variations. Each fetus was examined viscerally by fresh dissection and the sex verified. Kidneys were graded for renal papillae development by the method of Woo & Hoar (1972). Heads from approximately one-half

of the fetuses in each litter were placed in Bouin's fixative for subsequent section. The heads from the remaining one-half of the fetuses were examined by mid-coronal slice. All carcasses were eviscerated and processed for skeletal examination.

All animals survived to terminal sacrifice. At the highest dose, treatment-related clinical signs of toxicity included pale extremities, dried red material on the forelimbs or around the nose, decreased defecation, and dried brown vaginal discharge. Dams at 100 mg/kg bw per day also had an increase in the incidence of dried red material around the nose. Mean maternal body weights were significantly lower than those of the controls for the groups at the intermediate and highest doses beginning on day 8 and 7 of gestation, respectively, and continuing until termination. Final body weights of the groups at the intermediate and highest doses were 93% and 90%, respectively, of the level for the control group. The most pronounced effect on body weights of these treated groups was an overall weight loss during day 6–9 of gestation. Maternal food consumption by the groups at the intermediate and highest doses was significantly less than that by the control group throughout the dosing interval. No treatment-related effects were seen on reproductive indices of controls and treated groups.

No treatment-related effects were seen in the mean number of corpora lutea, implantation sites, pre- and postimplantation loss, or early and late resorptions. There were no statistically significant differences in litter size, number of fetuses, number of implantations, mean fetal weight, or fetal sex ratio. The number of early resorptions was increased at 100 and 500 mg/kg bw per day, with 10, 15, 25 and 19 fetuses (or 2.7, 3.9, 9.7 and 5.4%) resorbed. This was not dose-related, and was within the historical control range of 2.2-13.5%. Additionally, the control value at 2.7% was low, as only 5 out of 143 data sets in the historical control group had a resorption rate of less than 3%. Given this information, the increase in resorptions was not considered to be treatment-related. Postimplantation loss was significantly higher at 100 and 500 mg/kg bw per day compared with controls. No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetuses. The incidence rate of litters containing fetuses with malformations in the groups at 0, 10, 100, and 500 mg/kg bw per day was 0/25, 0/22, 1/24, and 2/24, respectively. There was one fetus at 100 mg/kg bw per day in one litter with umbilical herniation and one fetus from one litter at 500 mg/kg bw per day with umbilical herniation. These were within historical control range, and considered incidental. There were also two fetuses from one litter at 500 mg/kg bw per day with retroesophageal aortic arch, which was not considered to be treatment-related since it was only slightly above the historical control incidence and was seen in only one litter. Variations common to fetuses from treated and control litters included ossification of the cervical centrum, 14th rudimentary ribs, and unossified sternebrae.

The NOAEL for maternal toxicity was 10 mg/kg bw per day. The LOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of abnormal clinical signs, reduced body weights and body-weight gains and reduced food consumption. The NOAEL for developmental toxicity was 500 mg/kg bw per day, the highest dose tested (Schardein, 1997b).

Rabbits

In a range-finding study of developmental toxicity, five pregnant New Zealand White SPF rabbits per treatment group were given bifenazate (lot No. CPL00376RC; purity, 90.1%) at a dose of 0, 125, 250, 500, 750 or 1000 mg/kg bw per day (not adjusted for purity) by gavage in 0.5% carboxymethylcellulose on days 7–20 of gestation, inclusive. All animals were observed twice per day for mortality and moribundity and once per day for clinical signs of toxicity. Maternal body weights were recorded on days 0, 7, 10, 13, 16, 19, 21, 25, and 29 of gestation. 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, opened, and the location and number of viable and non-viable fetuses, early and late resorptions, and the total number of implantations were recorded. Uteri from females that appeared non-gravid were opened

and placed in 10% ammonium sulfide to detect any early implantation loss. Maternal tissues were preserved for histological examination only as indicated by gross findings. All fetuses were weighed and examined for external malformations/variations. Crown-rump measurements were recorded for late resorptions and the tissues were discarded. Each fetus was examined viscerally by fresh dissection and the sex determined. The brain from each fetus was examined by mid-coronal slice. All carcasses were eviscerated and processed for skeletal examination.

One control animal died due to gavage error. One animal at 750 mg/kg bw per day died on day 17 of gestation. Two animals were found dead, one on day 11 and one on day 21 of gestation at 1000 mg/kg bw per day. One animal at 500 mg/kg bw per day was euthanized in extremis on day 21 of gestation after the observation of prostration, rigid body, and loss of righting reflex. On postmortem examination of this rabbit, foci in the lungs and liver were found. Discoloured urine was observed in five out of five animals in all treatment groups. Decreased defaecation was observed at 250 mg/kg bw per day and above. Abortions were seen in 0, 0, 3, 2, 4 and 1 rabbit at 0, 125, 250, 500, 750 and 1000 mg/kg bw per day, respectively. Decreased body weights and body-weight gains were seen during the treatment at 500 mg/kg bw per day and above, with marginal effects at 250 mg/kg bw per day. The mean number of live fetuses were 3.6, 5.0, 3.0, 5.5, 0 and 3.3 at 0, 125, 250, 500, 750 and 1000 mg/kg bw per day respectively, with 5, 5, 1, 2, 1 and 3 pregnant rabbits examined in each of these groups, respectively. There were insufficient pups to allow an evaluation of developmental toxicity in this study.

On the basis of abortions at 250 mg/kg bw per day and above, the highest dose for the definitive study was suggested to be not more than 250 mg/kg bw per day (Denny, 1996).

In a study of developmental toxicity, groups of 20 pregnant New Zealand White rabbits were given bifenazate (lot No. PP159981-B; purity, 92.5%) at a dose of 0, 10, 50, or 200 mg/kg bw per day by gavage in 0.5% carboxymethylcellulose on days 7–19 of gestation, inclusive. Stability, homogeneity and dose concentrations were confirmed analytically. All animals were observed twice daily for mortality and moribundity and once daily for clinical signs of toxicity. Animals were also observed for signs of toxicity at approximately 1 h after dosing. Maternal body weights were recorded on day 0 of gestation, daily on days 7–20 of gestation, and on days 24 and 29. Food consumption was measured daily. A postmortem examination was done on all females aborting during the study. 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, opened, and the location and number of viable and non-viable fetuses, early and late resorptions, and the total number of implantations were recorded. Uteri from females that appeared non-gravid were opened and placed in 10% ammonium sulfide to detect any early implantation loss. Maternal tissues were preserved for histological examination only as indicated by gross findings. All fetuses were weighed and examined for external malformations/variations. Crown-rump measurements were recorded for late resorptions and the tissues were discarded. Each fetus was examined viscerally by fresh dissection and the sex determined. The brain from each fetus was examined by mid-coronal slice. All carcasses were eviscerated and processed for skeletal examination.

There were no deaths and no treatment-related clinical signs during the study. One doe in each of the groups at 0, 50, and 200 mg/kg bw per day aborted and was necropsied on day 21, 20, and 26 of gestation, respectively. These abortions were not considered to be treatment-related. No statistically or biologically significant differences in absolute body-weight changes occurred between the treated and control groups during the study. No statistically significant differences in food consumption were seen between the treated groups and the control group at any time during the study. No treatment-related findings were observed in any animals at necropsy. No treatment-related differences were observed between the treated and control groups for number of corpora lutea per doe, implantations per doe, pre- or postimplantation loss, resorptions per doe, fetal body weights, or fetal sex ratios. The slight (not significant) increase in early resorptions per doe in the group at the highest dose was due to

two animals with whole litter resorption which consisted of early resorptions of 1 and 7 implantation sites, respectively. This resulted in a corresponding increase (not significant) in postimplantation loss for the group at the highest dose. No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetuses.

The NOAEL was 200 mg/kg bw per day, the highest dose tested (Schardein, 1997a).

2.6 Special studies: studies on metabolites

No studies on toxicity of metabolites were available.

3. Observations in humans

A toxicological working paper on bifenazate prepared for the Meeting by Crompton Corporation stated that "monitoring of production plant personnel following normal safety precautions have not indicated any significant adverse effects". It also stated that "a review of incidental reports regarding bifenazate showed that the incidents were minor in nature and dissipated within a reasonable period of time. No oral studies have been performed with bifenazate in volunteers".

Comments

Biochemical aspects

In studies of toxicokinetics, rats were given a single dose of radiolabelled bifenazate (10 or 1000 mg/kg bw) or were pre-treated with unlabelled bifenazate (10 mg/kg bw per day for 14 days) then given a dose of radiolabelled bifenazate administered by gavage. On the basis of urinary and biliary excretion, approximately 79–85% and 22–29% of the orally administered dose was absorbed within 72 h at 10 and 1000 mg/kg bw. A peak in plasma concentrations of radioactivity was observed 5–6 h and 18–24 h after dosing at 10 and 1000 mg/kg bw, respectively. The elimination half-life at 10 and 1000 mg/kg bw was between 11.5 and 15.6 h. Approximately 90% of the administered dose was eliminated in excreta within 48 h and 96 h at 10 and 1000 mg/kg bw, respectively. Faeces were the major route of excretion (66–82% of the administered dose), with 8–24% of the administered dose being recovered in the urine. Approximately 68–73% of the administered dose was excreted in the bile within 72 h at 10 mg/kg bw and 21–26% of the administered dose at 1000 mg/kg bw. Approximately 0.5% of the administered dose was detected in the tissues and residual carcass at 168 h, with highest concentrations of radioactivity in the liver, kidney and blood. Systemically absorbed bifenazate was extensively metabolized. The major metabolites of bifenazate in the faeces and urine resulted from hydrazine oxidation, demethylation, ring hydroxylation, removal of the hydrazine-carboxylic acid side-chain, and conjugation of the biphenyl ring moiety with glucuronic acid and sulfate. A total of eight metabolites and bifenazate were identified in the faeces and three metabolites were identified in the urine. The primary metabolite(s) in the urine were sulfate and glucuronide conjugates. Unchanged bifenazate was isolated in the faeces of males and females (5–7% and 48–61% of the faecal radioactivity at 10 and 1000 mg/kg bw, respectively). In faeces, metabolite D3598 (a product of oxidation of the hydrazine moiety) was detected (4–5% and 1–2% of the faecal radioactivity at 10 and 1000 mg/kg bw, respectively). Excretion, distribution and metabolite profiles were essentially independent of pre-treatment and sex.

Toxicological data

Bifenazate has low toxicity when administered orally, dermally or by inhalation. The LD_{50} after oral administration was > 5000 mg/kg bw in mice and rats. The LD_{50} in rats treated dermally

was > 5000 mg/kg bw. The LC_{50} in rats treated by inhalation (nose only) was > 4.4 mg/l (dust). Bifenazate was slightly irritating to the eyes and skin of rabbits. Bifenazate was not a skin sensitizer in guinea-pigs (Buehler test), but gave a positive response (mild sensitizer) in a Magnusson & Kligman (maximization) test in guinea-pigs.

In the absence of any specific studies addressing effects after single doses, attention was paid to effects after one or several doses in short-term studies with repeated doses. In a 28-day feeding study in mice, there were no deaths at 200 ppm (equivalent to 34 mg/kg bw per day). Deaths were observed starting on day 3 at 1000 ppm and above. At a dose of 2500 or 5000 ppm, all treated animals died. Antemortem findings (hunched posture, hypoactive behaviour, pale appearance, urine staining, tremors, dyspnoea, thinness and/or partial eye closure) were not reported before days 6–8 in 5 out of 20 animals at approximately 275 mg/kg bw per day, or before days 7–8 in 2 out of 10 males at approximately 550 mg/kg bw per day. In a 28-day feeding study in rats, there were no deaths before dosing day 14 in animals given bifenazate at a dose of up to 10 000 ppm (equal to 410 mg/kg bw per day) and there were no clinical signs before week 2 of dosing at up to 10 000 ppm. Toxicologically significant reductions in erythrocyte counts, haemoglobin and erythrocyte volume fraction were observed at 10 000 ppm at termination.

In short-term and long-term studies in mice, rats and dogs, the primary effects of bifenazate were on the haematopoietic system and the liver.

In a 90-day dietary study of toxicity in mice, an increase in the incidence of haemosiderin pigment and/or severity of this effect was seen in the spleen at 100 ppm (equal to 16.2 mg/kg bw per day) and above, although no significant changes in blood parameters were seen. The NOAEL was 50 ppm (equal to 8.0 mg/kg bw per day).

In a 90-day dietary study of toxicity in rats, decreased body-weight gains (females), decreases in erythrocytes and haemoglobin (females), increases in relative spleen and kidney weights (females), hepatocellular necrosis (males), increased pigment in the spleen (both sexes) extramedullary haematopoiesis in the spleen (females), vacuolation of the zona fasciculata of the adrenal cortex (males) and hepatocellular hypertrophy were seen at 200 ppm (equal to 13.3 mg/kg bw per day). The NOAEL was 40 ppm (equal to 8.0 mg/kg bw per day).

In a 90-day study of toxicity in dogs, alterations in haematological and related parameters (decreases in erythrocytes, haemoglobin and erythrocyte volume fraction, increases in reticulocytes, MCV, MCH, anisocytosis and platelets, a decrease in protein peak 4 (females only), brown-coloured urine (males only), brown pigment in Kupffer cells), increases in absolute and relative liver weights and hepatocellular hypertrophy (females only) were seen at 400 ppm (equal to 10.4 mg/kg bw per day). The NOAEL was 40 ppm (equal to 0.9 mg/kg bw per day). In a 52-week study of toxicity in dogs, haematological changes (decreases in erythrocyte counts, haemoglobin and erythrocyte volume fraction; increases in reticulocyte counts and MCV), changes in clinical chemistry and urine analysis parameters (increased concentration of serum total bilirubin, urinary bilirubin, and brown coloration of the urine), and histopathological changes (mild to moderate hyperplasia of the bone marrow of the rib, femur and sternum and brown pigment in liver and kidney) were seen at the LOAEL of 400 ppm (equal to 8.9 mg/kg bw per day). The NOAEL was 40 ppm (equal to 1.01 mg/kg bw per day).

When administered dermally, bifenazate displayed toxic effects that were qualitatively similar to those seen after oral administration. In a 21-day study of dermal toxicity in rats, the NOAEL was 80 mg/kg bw per day based on haematological effects seen at 400 mg/kg bw per day and above.

Bifenazate gave negative results in an adequate range of studies of genotoxicity in vitro and in vivo.

The Meeting concluded that bifenazate is unlikely to be genotoxic.

In long-term studies of toxicity and carcinogenicity in mice and rats, there was no treatment-related neoplasticity. Survival was not affected by treatment with bifenazate in mice and rats. At

100 ppm (equal to 19.7 mg/kg bw per day), decreases in counts for leukocytes and lymphocytes were observed in male mice. In female mice, decreases in body-weight gains were observed at 175 ppm (equal to 35.7 mg/kg bw per day). The NOAEL was 10 ppm (1.5 mg/kg bw per day) in males and 100 ppm (equal to 19.7 mg/kg bw per day) in females. In rats, decreases in body-weight gain, food consumption and haematological parameters were seen at the LOAEL of 200 ppm (equal to 9.7 mg/kg bw per day). The NOAEL for systemic toxicity was 80 ppm (equal to 3.9 mg/kg bw per day). Bifenazate was not carcinogenic in mice or rats.

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

In a two-generation study of reproduction in rats, offspring toxicity or reproductive parameters were not affected at the highest dose tested (200 ppm, equal to 15.3 mg/kg bw per day). The NOAEL for parental systemic toxicity was 20 ppm (equal to 1.5 mg/kg bw per day) on the basis of decreases in body-weight gains. Bifenazate was not embryotoxic, fetotoxic or teratogenic at doses of 500 (the highest dose tested) or 200 mg/kg bw per day (the highest dose tested) in rats and rabbits, respectively.

The Meeting concluded that bifenazate is neither teratogenic nor a reproductive toxicant.

No treatment-related clinical signs of neurotoxicity were observed in the studies that were provided. Therefore, no specific studies of neurotoxicity were necessary.

No studies of toxicity with metabolites of bifenazate were submitted. Parent bifenazate and bifenazate-diazene (D 3593) readily undergo chemical interconversion, so the Meeting considered that these compounds were assessed in the studies with bifenazate. Since other major metabolites of bifenazate are polar glucuronide or sulfate conjugates that are rapidly excreted, the Meeting concluded that these metabolites are likely to be less toxic than bifenazate.

No significant adverse effects were reported in personnel of production plants.

The Meeting concluded that the existing database on bifenazate 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 based on a NOAEL of 40 ppm (equal to 1.0 mg/kg bw per day) for compensatory haematopoiesis, alteration in urine analysis parameters and liver toxicity seen at 400 ppm (equal to 9.0 mg/kg bw per day) and above in a 52-week study in dogs fed bifenazate, and using a 100-fold safety factor.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for bifenazate on the basis of its low acute toxicity, lack of haemolytic effects in a 28-day study of toxicity in mice, the absence of developmental toxicity in rats and rabbits, the lack of clinical signs of neurotoxicity in the database, and the absence of any other toxicological end-point that would be likely to be elicited by a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	79-week study of toxicity and carcinogenicity[a]	Toxicity	10 ppm, equal to 1.5 mg/kg bw per day	100 ppm, equal to 15.4 mg/kg bw per day
		Carcinogenicity	175 ppm, equal to 35.7 mg/kg bw per day[c]	
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	80 ppm, equal to 3.9 mg/kg bw per day	160 ppm, equal to 9.7 mg/kg bw per day

		Carcinogenicity	160 ppm, equal to 9.7 mg/kg bw per day[c]	—
	Multigeneration study of reproductive toxicity[a]	Parental toxicity	20 ppm, equal to 1.5 mg/kg bw per day	80 ppm, equal to 6.1 mg/kg bw per day
		Offspring toxicity	200 ppm equal to 15.3 mg/kg bw per day	
	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	100 mg/kg bw per day
		Embryo/fetotoxicity	500 mg/kg bw per day[c]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity	200 mg/kg bw per day[c]	—
		Embryo-fetotoxicity	200 mg/kg bw per day	—
Dog	90-day study of toxicity[a]	Toxicity	40 ppm, equal to 0.9 mg/kg bw per day	400 ppm, equal to 10.4 mg/kg bw per day
	One-year study of toxicity[a]	Toxicity	40 ppm, equal to 1.0 mg/kg bw per day	400 ppm, equal to 8.9 mg/kg bw per day

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

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 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 bifenazate

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Moderate and incomplete; maximum blood concentration reached by 5–6 h; later at higher doses. Approximately 79–85% and 22–29% absorbed within 72 h at 10 and 1000 mg/kg bw, respectively
Distribution	Widely distributed in tissues
Potential for accumulation	No evidence of significant accumulation
Rate and extent of excretion	Approximately 90% (27% in urine and 63% in faeces) within 48 h at 10 mg/kg bw per day
Metabolism in animals	Extensive; metabolic pathways include hydrazine oxidation, demethylation, ring hydroxylation, cleavage of the hydrazine-carboxylic acid portion of the molecule and conjugation with glucuronic acid and sulfate
Toxicologically significant compounds	Bifenazate and bifenazate-diazene (compounds readily interconvert)

Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 4.4 mg/l dust (4-h exposure, nose only)
Rabbit, skin irritation	Minimal irritation
Rabbit, eye irritation	Minimal irritation
Guinea-pig, skin sensitization	Not a sensitizer (Buehler test) Mild sensitizer (maximization)
Short-term studies of toxicity	
Target/critical effect	Haematopoietic system
Lowest relevant oral NOAEL	1.0 mg/kg bw per day (90-day and one-year study in dogs)
Lowest relevant dermal NOAEL	80 mg/kg bw per day (21-day study in rats)
Lowest relevant inhalation NOAEL	No data
Genotoxicity	
	No genotoxic potential
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Haematopoietic system
Lowest relevant NOAEL	1.5 mg/kg bw per day (78-week study in mice)
Carcinogenicity	Not carcinogenic in mice and rats
Reproductive toxicity	
Reproduction target/critical effect	No toxicologically relevant effects
Lowest relevant reproductive NOAEL	15.3 mg/kg bw per day (rats, highest dose tested)
Developmental target/critical effect	No developmental toxicity in rats and rabbits at highest dose tested
Lowest relevant developmental NOAEL	200 mg/kg bw per day (rabbits; highest dose tested)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	No clinical signs observed in available toxicological studies

Medical data

No significant adverse health effects reported

Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Dog, one-year study of toxicity	100
ARfD	Unnecessary	—	—

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BOSCALID

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Explanation

Boscalid is the provisionally approved International Organization of Standardization (ISO) name for 2-chloro-*N*-(4'-chlorobiphenyl-2-yl)nicotinamide (International Union of Pure and Applied Chemistry, IUPAC) or 2-chloro-*N*-(4'-chloro[1,1'-biphenyl]-2-yl)-3-pyridinecarboxamide (Chemical Abstracts Service, CAS). It is an anilide fungicide that inhibits mitochondrial respiration, thereby inhibiting spore germination, germ-tube elongation, mycelial growth, and sporulation of pathogenic fungi on the leaf surface, and is used against a broad spectrum of diseases in a wide range of crops. Boscalid was reviewed for the first time by the present Meeting at the request of the Codex Committee on Pesticide residues (CCPR). All critical studies complied with good laboratory practice (GLP).

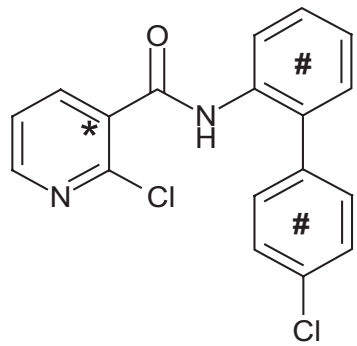
Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Studies of metabolism in rats treated orally were conducted with [diphenyl-ring-U-^{14}C]boscalid (batch Nos 641-1018 and 641-1101; purity, > 98%, radiochemical purity, > 95%), [pyridin-ring-3-^{14}C] boscalid (batch No. 640-1026; purity, > 97%; radiochemical purity, > 95%) and nonradiolabelled boscalid (batch No. 01174-236; purity > 99%). The positions of the radiolabel are shown in Figure 1.

Figure 1. Structure and position of ^{14}C-labelled boscalid



= [diphenyl-U-^{14}C]boscalid
* = [pyridine-3-^{14}C]boscalid

The absorption, distribution, elimination and kinetics of ^{14}C-labelled boscalid were investigated in male and female Wistar rats given doses of 50 and 500 mg/kg bw. The experiments were performed with boscalid labelled in the diphenyl ring. An additional experiment investigating the balance and excretion pattern was performed with boscalid labelled in the pyridine ring.

Tissue distribution was determined using groups of 12 male and 12 female rats for each dose. For all other experiments in vivo, groups of four male and four female rats were used.

The stability, homogeneity and correctness of the test substance preparation were analytically verified.

The results of these experiments are summarized in Table 1. There was no radioactivity in the expired air after the administration of the diphenyl- or pyridine-ring label at a dose of 500 mg/kg bw. Within 168 h after administration of a single oral dose at 500 mg/kg bw (diphenyl label), 3% of the administered radioactivity was excreted in the urine of males and females and 91% (males) and 97% (females) was found in the faeces. Radioactivity remaining in tissues and organs 168 h after dosing was less than 1 µg eq/g at a dose of 500 mg/kg bw, except in the gastrointestinal tract.

Virtually the same excretion pattern was observed as with the diphenyl label after the administration of the pyridine label as a single oral dose at 500 mg/kg bw and after repeated oral administration of diphenyl-labelled boscalid (14 doses of unlabelled boscalid at 500 mg/kg bw, one dose of labelled boscalid at 500 mg/kg bw).

Within 168 h after a single oral dose of diphenyl-labelled boscalid at 50 mg/kg bw, 16% of the administered radioactivity was excreted in the urine of males and females and 85% (males) and 79% (females) was found in the faeces. Radioactivity remaining in tissues and organs 168 h after dosing was less than 0.3 µg eq/g at a dose of 50 mg/kg bw, except in the gastrointestinal tract. The overall recovery of radioactivity was in the range of 93.9–102.8% in all experiments.

Within 48 h after administration of [^{14}C]boscalid at a dose of 500 mg/kg bw, 11% (males) and 12% (females) of the administered radioactivity was excreted via bile, while after administration of 50 mg/kg bw of [^{14}C]boscalid, 39% (males) and 40% (females) of the administered radioactivity was excreted via bile. These percentages are considerably lower than the 70–90% dose recovery in faeces. The difference indicates that boscalid is not completely absorbed from the gastrointestinal tract.

Table 1. Excretion balance (as a percentage of administered radioactivity) at 168 h after oral dosing and biliary excretion after 48 h

	Excretion balance (percentage of administered radiobel)			
	Dose (mg/kg bw)			
	500	50	500	500
	Diphenyl-labelled	Diphenyl-labelled	Diphenyl-labelled	Pyridine-labelled
	Single dose[a,b]	Single dose[a,b]	Repeated doses	Single dose
Males				
Urine 0–168 h	2.73	16.38	2.64	5.21
Cage wash	0.41	0.26	0.11	0.10
Faeces 0–168 h	90.69	84.86	94.88	89.61
Carcass + organs	0.05	0.04	0.11	0.04
Total	93.87	101.54	97.74	94.96
Bile 0–48 h	10.69	39.29	—	—
Females				
Urine 0–168 h	2.88 (5.67)	15.72 (25.74)	3.99	3.80
Cage wash	0.04 (0.12)	1.03 (0.03)	0.18	0.30
Faeces 0–168 h	97.35 (87.26)	79.27 (80.50)	98.47	92.15
Carcass + organs	0.02 (0.05)	0.04 (0.07)	1.10	0.02
Total	100.29 (93.10)	96.06 (106.34)	102.75	96.27
Bile 0–48 h	11.93	39.92	—	—

[a] Experiments with females were repeated for the lowest and highest doses because of evidence that some faecal samples had been mislabelled. These data were not used in the calculation of kinetic parameters.
[b] Numbers in parenthese are results for the repeated experiments.

If it is assumed that the amount of radioactivity excreted in bile and urine represents the amount of [^{14}C]boscalid that is bioavailable, then bioavailability at the lowest dose (50 mg/kg bw) is approximately 50%.

At both doses, the changes in radioactivity concentration with time were similar in blood cells and in plasma. During the first 24 h after dosing, lower concentrations of radioactivity were found in blood cells than in plasma, while at later times this relationship was reversed in the groups at the highest dose (Table 2).

Table 2. Radioactivity in blood cells and plasma of rats given a single dose of [^{14}C]boscalid (diphenyl label)

Tissue	Concentration of radioactive residues (µg eq/g tissue)											
	Dose (mg/kg bw)											
	50						500					
	Males			Females			Males			Females		
	Time-point (h)											
	8	24	35	8	24	35	8	24	35	8	24	35
Blood cells	0.64	0.12	NA	0.78	0.13	NA	1.74	0.31	0.22	1.35	0.43	0.13
Plasma	1.14	0.14	NA	1.52	0.17	NA	3.52	0.40	0.09	2.84	0.51	0.17

From Leibold et al. (2000) and Leibold (2002)
NA, not available.

In rats given a single oral dose of [^{14}C]boscalid at 500 mg/kg bw, the plasma concentration–time curve showed two maxima. The first plasma maximum was reached 0.5–1 h after dosing with concentrations of 2.61 µg eq/g in males and 3.52 µg eq/g in females. The second plasma maximum occurred after 8 h, when plasma concentrations were 4.46 and 3.77 µg eq/g in males and females, respectively. At later times, plasma concentrations declined to 0.01 µg eq/g in males and 0.03 µg eq/g in females at 120 h after dosing. The initial half life (t½) was calculated to be 8.0 h in males and 9.1 h in females. The terminal t½ was 20.2 h in males and 27.4 h in females. The area under the concentration–time curve (AUC) was 68.4 µg eq × h/g in males and 75.5 µg eq × h/g in females.

In rats given a single oral dose of 50 mg/kg bw of [^{14}C]boscalid, the plasma concentration–time curve also showed two maxima. The early peak was reached after 0.5 h when plasma concentrations were 0.99 µg eq/g in males and 1.40 µg eq/g in females. The later peak occurred after 8 h, when plasma concentrations were 1.54 and 1.58 µg eq/g in males and females, respectively. After this second maximum, there was a biphasic decline in plasma concentrations to 0.01 µg eq/g in both males and females at 120 h after dosing. The initial t½ values were 7.2 h and 8.2 h in males and females, respectively. Terminal t½ values were 41.7 and 30.1 h in male and female rats, respectively. The AUC values were 21.2 µg eq × h/g in males and 24.4 µg eq × h/g in females. Increasing the dose by a factor of about 10 thus resulted in an increase of the AUC values by a factor of approximately 3 in both sexes. These kinetic data are summarized in Table 3.

Table 3. Pharmacokinetic parameters of radioactivity in plasma after single oral doses of [^{14}C]boscalid (diphenyl label)

Parameter	Dose (mg/kg bw)			
	50		500	
	Males	Females	Males	Females
First C_{max} (µg eq/g)	0.99	1.40	2.61	3.52
First T_{max} (h)	1.0	0.5	0.5–1.0	0.5–1.0
Second C_{max} (µg eq/g)	1.54	1.58	4.46	3.77
Second T_{max} (h)	8	8	8	8
Initial t½ (h)	7.2	8.2	8.0	9.1
Terminal t½ (h)	41.7	30.1	20.2	27.4
AUC (µg eq × h/g)	21.2	24.4	68.4	75.5

From Leibold et al. (2000) and Leibold (2002)
AUC, area under the curve of concentration–time.

After a single oral dose of [^{14}C]boscalid at 500 mg/kg bw, tissue radioactivity concentrations were measured 8, 18, 24 and 35 h after dosing. At the lowest dose of 50 mg/kg bw, the corresponding radioactivity measurements were made 8, 17, 21 and 24 h after dosing. In general, tissue radioactivity concentrations in both sexes were in the same range at the respective time-points and doses. The pattern of distribution and elimination in various organs and tissues was also similar. Tissue radioactivity concentrations declined with time and at a similar rate to the plasma concentrations. Throughout the time-course of the experiments, the highest concentrations of radioactivity were found in the gastrointestinal tract and liver. Concentrations of radioactivity were lowest in the brain. No evidence for a cumulative potential could be deduced from the available data (Leibold et al., 2000; Leibold, 2002).

Studies of metabolism in rats treated by application to the skin were conducted using [diphenyl-ring-U-^{14}C]boscalid (batch No. 641-2017; radiochemical purity, > 98% and non-radiolabelled boscalid (batch No. 01183-190; purity, 99.3%).

The absorption, distribution and excretion of radioactivity was studied in male Wistar rats given a single dermal administration of [^{14}C]boscalid mixed with non-labelled boscalid and taken up in water at nominal doses of 0.01, 0.10 and 1.00 mg/cm^2 corresponding to 0.1, 1.0 and 10 mg/rat or about 0.4, 4 and 40 mg/kg bw. Groups of four animals were exposed according to the regimen described in Table 4.

Table 4. Design of an experiment in rats given [^{14}C]boscalid as a single dermal application

Duration of exposure (h)	1	4	10	24	10[a]	10
Rats killed after (h)	1	4	10	24	24[a]	72

From Leibold & Hoffmann (2001)

[a] Only at the lowest dose.

Twenty-four hours before dosing, an intrascapular area (about 10 cm^2) of each rat was clipped free of hair and washed with acetone. A silicone ring was glued to the skin and the test substance preparation (about 10 µl/cm^2) was applied within the ring from a syringe that was weighed before and after application. A nylon mesh was then glued to the surface of the silicone ring and a porous bandage used to encircle the trunk of the rat. The rats (four per group) were dosed and then placed in metabolism cages in order to collect excreta for up to 72 h.

After the required exposure period, the protective cover was removed and the exposed skin was washed with a mild soap solution. At the end of the various collection periods, rats were killed and the following specimens/tissues were assayed for remaining radioactivity: excreta, blood cells, plasma, liver, kidneys, carcass, treated skin (application site) and non-treated areas (surrounding skin). For balance estimates, the cage wash, skin wash and the protective cover (including the silicone ring) were also assayed for radioactivity. The results are given in Table 5.

The radioactivity absorbed was excreted mainly in the faeces. Due to the very low skin penetration, concentrations of radioactivity in organs and tissues analysed were also very low. Low amounts of the radioactivity (0.15–3.76%) remained in the skin at the application site at the end of exposure.

Table 5 Radioactivity absorbed (percentage of administered dose and total amount) by rats given [^{14}C]boscalid as a single dermal application

Exposure time (h)	Sacrifice time (h)	Dose (mg/cm^2)					
		1.00		0.1		0.01	
		% absorbed	mg/animal	% absorbed	mg/animal	% absorbed	mg/animal
1	1	0.17	0.0184	0.37	0.0046	0.52	0.0005
4	4	0.33	0.0250	0.25	0.0032	2.02	0.0025

10	10	0.42	0.0462	0.63	0.0080	8.07	0.0102
10	24[a]	—	—	—	—	6.26	0.0070
10	72	1.48	0.1364	2.07	0.0262	5.72	0.0065
24	24	0.41	0.0391	2.63	0.0343	10.93	0.0143

From Leibold & Hoffmann (2001)
[a] Only at the lowest dose.

The dosing regime most similar to a worst-case situation during work in which the operator did not wash his hands, was an exposure of 10 h. The proportions of applied radioactivity that were absorbed through the skin during this period of exposure at a dose of 0.01, 0.1 and 1.0 mg/cm^2, respectively, were 8.07%, 0.63% and 0.42%. A group of rats receiving the lowest dose was killed 24 h after the start of the 10 h exposure. In this group, the proportion absorbed was 6.26%. Thus, an average (worst-case) dermal penetration of 8% can be assumed (Leibold & Hoffmann, 2001).

The penetration of [diphenyl-ring-U-^{14}C]boscalid (batch No. 641-2017; radiochemical purity, > 98% and non-radiolabelled boscalid (batch No. 01183-190; purity, 99.3%) through isolated rat and human epidermal preparations in vitro was determined after 24 h exposure to boscalid at a concentration of 10, 100 or 1000 µg/cm^2. On the day before application of the dose, the integrity of the epidermal membranes and their suitability for use in the study was assessed by measuring the penetration of tritiated water applied to the membranes and comparison of the results with exclusion criteria. After this membrane integrity check and before dose application, the receptor chamber was refilled with ethanol : water (1 : 1 v/v). The receptor fluid was chosen on the basis that boscalid is soluble in ethanol.

The dose formulation was applied to the upper surface of the epidermal membranes using a positive displacement pipette. The amount of solution applied to each membrane was calculated by weight difference of the positive-displacement pipette before and after dose administration. Duplicate 0.1 ml samples of receptor fluid were taken immediately before dosing and at 0.5, 1, 2, 4, 6, 10 and 24 h after addition of the formulation. An equal volume of fresh receptor fluid was added to the receptor chamber after each sampling occasion, excluding the final sample time, in order to maintain a constant volume of receptor fluid in the receptor chamber of the diffusion cell.

Twenty-four hours after the last receptor fluid sample was taken, the receptor fluid was removed from the receptor chambers of all cells and retained. Any residual formulation was washed from the surface of the skin with a soap solution without organic solvent and rinsed with deionized water. The washings were retained for analysis. The membrane preparations were removed from the cells and homogenized. All washings from the dismantled cell were retained. Radioactivity was measured in the receptor fluid, skin section, skin washings and apparatus washings. The percentage of the applied dose in each of the samples and the rate of penetration through the epidermal membranes (µg equivalents/cm^2 per h) were calculated. The stability, homogeneity and correctness of the test substance preparation were analytically verified.

Recoveries at 24 h were 99%, 96% and 106% for the rat epidermal membranes and 98%, 96% and 98% for the human epidermal membranes exposed to boscalid at 1, 10 and 100 mg/ml, respectively. The permeability coefficients (the ratio of the rate of penetration in µg equivalents/cm^2 per h to the difference in test compound concentration across the membrane × 10^{-5} cm/h) were 51.6,

Table 6. Transmembrane migration of boscalid in rat and human epidermis in vitro

Time (h)	Mean cumulative absorption of [^{14}C]boscalid (μg/cm^2 skin)					
	Administered dose (μg/cm^2)					
	10		100		1000	
	Rat	Human	Rat	Human	Rat	Human
0.5	0.264	0.002	0.302	0.009	NA	NA
1	0.462	0.014	0.657	0.032	7.703	1.404
2	0.691	0.029	1.178	0.108	11.47	1.929
4	0.977	0.046	1.667	0.157	13.03	2.026
6	1.143	0.061	1.890	0.204	15.63	2.143
10	1.366	0.103	2.593	0.304	20.64	4.295
24	1.906	0.264	3.650	1.320	27.87	5.283
Lag time (h)	0.055	0.185	0.110	0.227	0.219	0.115
Mean rate of penetration (μg/cm^2 per h)	0.302	0.015	0.685	0.050	3.813	0.498
Permeability coefficient (× 10^{-5} cm/h)	51.6	2.572	7.188	0.53	6.571	0.858

From Thornley & Bryson (2001)
NA, not applicable.

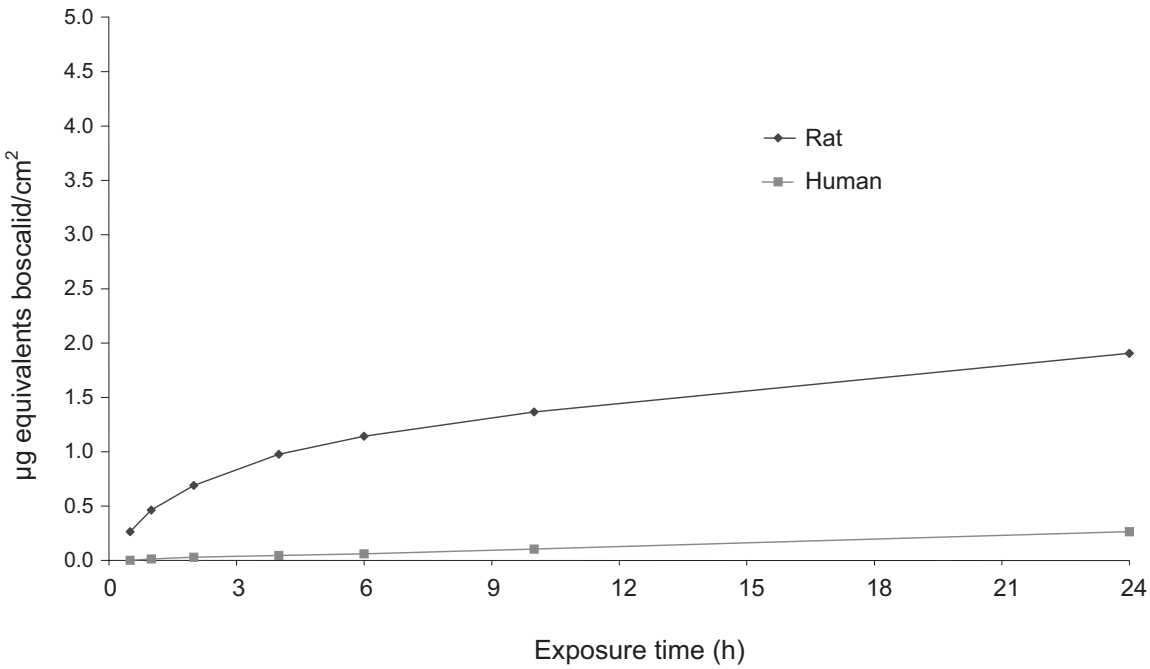
7.2 and 6.6 for rat epidermal membranes and 2.6, 0.53 and 0.86 for human epidermal membranes exposed at 10, 100 and 1000 μg/cm^2, respectively (Table 6).

Transfer of radioactivity across rat epidermal membranes was rapid during the early sampling times of the experiment, with a lag time at all doses of less than 14 min. Thereafter, the amount of radioactivity transferred decreased with time, the transfer rate between 10 h and 24 h being less than 14% of the initial rate. At the end of the study, the radioactivity transferred was 33%, 4% and 3% of the 10, 100 and 1000 μg/cm^2 doses, respectively. At the lowest, intermediate and highest doses, respectively, 31%, 73% and 96% of the applied radioactivity was recovered in the skin washings and was therefore unabsorbed. For a 10- and a 100-times increase in the concentration of the formulation applied to rat membranes, a corresponding 2.18- and 12.63-times increase in the initial rate of absorption was apparent.

Radioactivity was absorbed through human epidermal membranes within 14 min of application, regardless of the concentration of formulation. As with the rat, there was evidence to suggest an initial high rate of absorption within the first 4 h for some human epidermal membranes. The rate of absorption increased 3.3-fold and 33.2-fold for a 10- and 100-times increase in the concentration of boscalid.

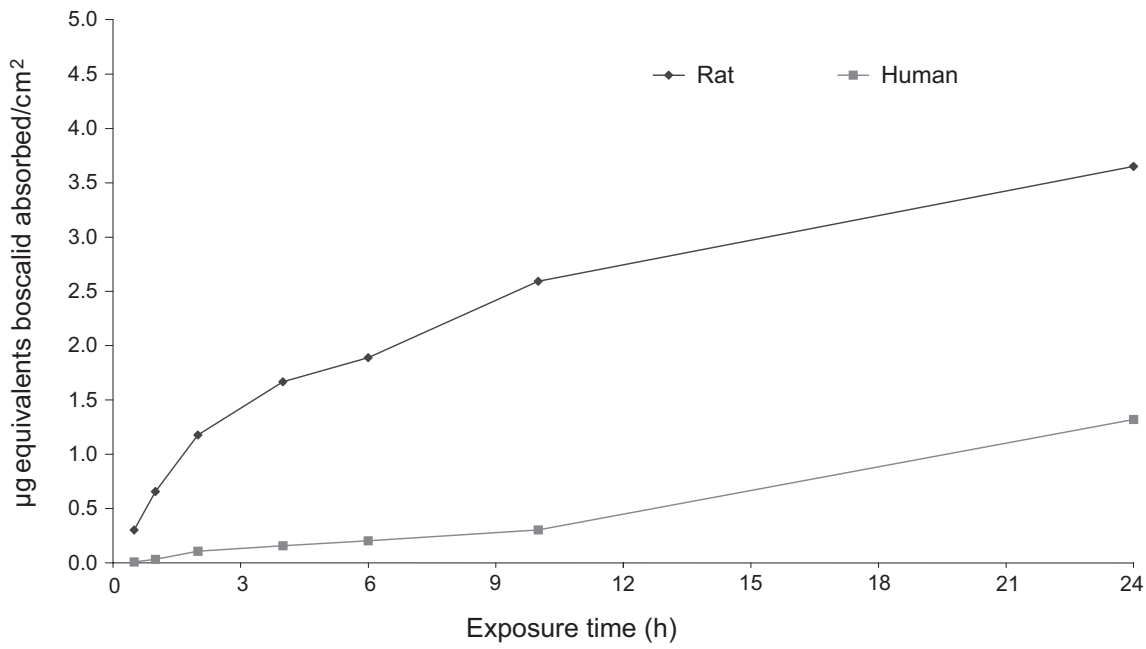
The time-courses of transfer across rat and human epidermal membranes at each concentration of boscalid are shown in Figures 2–4.

Figure 2. Transfer of radioactivity across rat and human epidermal membranes after a single application of [^{14}C]boscalid at a nominal dose of 10 µg/cm^2 (1 mg/ml)



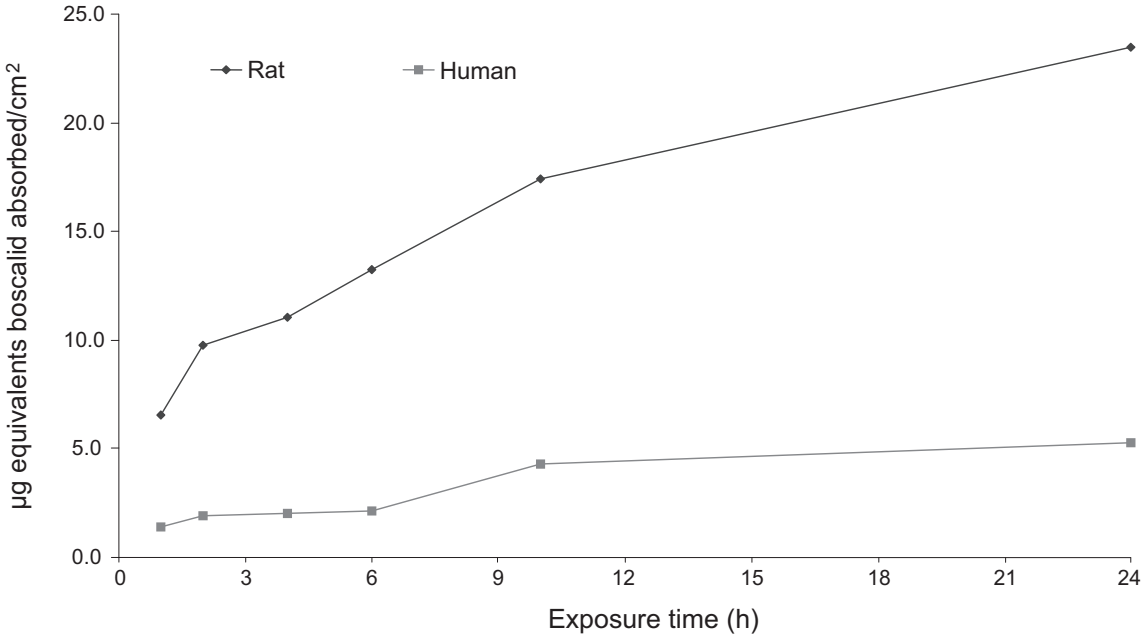
From Thornley & Bryson (2001)

Figure 3. Transfer of radioactivity through rat and human epidermal membranes after a single application of [^{14}C]boscalid at a nominal dose of 100 µg/cm^2 (10 mg/ml)



From Thornley & Bryson (2001)

Figure 4.Transfer of radioactivity through rat and human epidermal membranes after a singlapplication of [^{14}C]boscalid at a nominal dose of 1000 µg/cm^2 (100 mg/ml)



From Thornley & Bryson (2001)

The differences in the skin penetration between human and rat skin at the different doses (based on the rates of penetration) are shown in Table 7. Based on comparison of penetration rates or permeability coefficients, it appears that penetration rate through rat epidermis is at least a 7.7-fold that through human epidermis. The recovery of radiolabel was determined at the end of the experiment and summarized in Table 8 (Thornley & Bryson, 2001).

Table 7. Difference in penetration of boscalid through rat and human skin

Species	Mean rate of penetration (µg/cm^2 per h)		
	Dose administered (µg/cm^2)		
	10	100	1000
Rat epidermis	0.302	0.685	3.813
Human epidermis	0.015	0.050	0.498
Fold difference	20.1	13.7	7.7

From Thornley & Bryson (2001)

Table 8. Study of dermal penetration in vitro: recovery of radioactivity through rat and human skin

Parameter	Recovery of ^{14}C radiolabel (% of applied dose) at 24 h								
	Dose applied								
	0.01 mg/cm^2 (1 mg/ml)			0.1 mg/cm^2 (10 mg/ml)			1.00 mg/cm^2 (100 mg/ml)		
	Rat	Human	Ratio	Rat	Human	Ratio	Rat	Human	Ratio
Receptor fluid	32.54	4.545	7.2	3.954	1.416	2.8	3.482	0.641	5.4
Receptor cell wash	1.809	0.485	3.7	0.368	2.596	0.1	1.150	0.509	2.3
Total penetrated	*34.35*	*5.03*	*6.8*	*4.322*	*4.012*	*1.1*	*4.632*	*1.15*	*4.0*

Skin	33.07	61.59	0.5	17.94	17.29	1.0	4.366	1.086	4.0
Total penetrated + skin	*67.42*	*66.62*	*1.0*	*22.26*	*21.3*	*1.0*	*8.998*	*2.236*	*4.0*
Surface washings	31.28	31.16	1.0	73.06	73.45	1.0	96.33	95.30	1.0
Donor cell wash	0.635	0.309	2.1	1.014	1.806	0.6	0.376	0.299	1.3
Total	*99.34*	*98.09*	*1.0*	*96.33*	*96.39*	*1.0*	*105.70*	*97.84*	*1.1*

From Thornley & Bryson (2001)

The total amount of radiolabel that fully penetrated the skin membrane ("absorbed radiolabel") was recovered in the receptor fluid and in the wash of the receptor cell. At the lowest and highest concentrations, the amount of absorbed radiolabel was approximately sevenfold and fourfold higher in specimens from rats than from humans, while at the intermediate concentration, the absorption ratio was approximately 1. When the amount of radioactivity remaining associated with the skin was also taken into account, no differences in the "potentially absorbable" percentages of administered radiolabel were observed between species in the groups at the lowest and intermediate doses, while a fourfold higher recovery was established in the group at the highest dose.

Therefore, it can be concluded that absorption through rat epidermal membranes in vitro was the same as through human epidermal membranes at the lowest and intermediate doses (0.01 and 0.1 mg/cm^2) and approximately fourfold that at the highest concentration (1.00 mg/cm^2). The results obtained for the lowest and intermediate concentrations were considered to be the most relevant, because these concentrations fall in the range of the expected operator exposure. Thus, based on the results of the study of dermal penetration in vitro, is the Meeting concluded that there is no notable difference between rats and humans in the extent of bioavailability after dermal exposure to boscalid.

Metabolism

Studies of metabolic transformation (Grosshans & Knoell, 2001) were conducted in rats given [diphenyl-ring-U-^{14}C]boscalid (batch No. 641-2018; radiochemical purity, > 99%, specific activity, 5.23 MBq/mg) or [pyridine-3-^{14}C]boscalid (batch No. 640-1026; radiochemical purity, > 97%, specific activity, 5.81 MBq/mg) or non-radiolabelled boscalid (batch No. 01174-236; purity, > 99.4%). Male and female Wistar rats were orally dosed with [^{14}C]boscalid at a nominal dose of 50 and 500 mg/kg bw.

Patterns of radioactive metabolites in excreta (urine, faeces, bile), plasma, and tissues (liver and kidney) were analysed by high-performance liquid chromatography (HPLC). Metabolites were identified by mass spectroscopy (LC-MS and LC-MS/MS) and, in some cases, nuclear magnetic resonance analysis of isolated fractions. Metabolite patterns of samples generated in this study were compared with those obtained in kinetic studies in which rats were given single oral high (500 mg/kg) and low (50 mg/kg) doses and repeated high doses (14 × non-radiolabelled, 1 × radiolabelled; 500 mg/kg). The structures of the identified metabolites recovered from rat excreta, plasma and tissues are given in Table 19.

After administration of an oral dose of [^{14}C]boscalid to male and female rats, a large number of metabolites were detected in urine. Predominant metabolites were a 4-hydroxyl diphenyl ring metabolite (named M510F01 in the tables) and its glucuronic acid conjugate (named M510F02 in the tables). The proportion of the 4-hydroxy-diphenyl metabolite excreted via urine ranged from 0.5% to 3% in the group at 500 mg/kg bw and from 10% to 16% in the group at 50 mg/kg bw, while the proportion of its glucuronyl conjugate was in the range of 0.1–4% of the dose. Two more

metabolites found in the urine at up to 2% of the administered dose were formed by conjugation of glucuronide (M510F48) and cysteine (M510F05) at the expense of chlorine in the pyridine moiety. Minor metabolites identified in urine are identified in Figure 5 and Table 19 as M510F03, M510F04, M510F12, M510F20, and M510F42. Traces of parent substance could be detected. Metabolites found in the group receiving pyridine-labelled boscalid included traces of 2-chloronicotinic acid (M510F47), while 4-chloro-2-aminobiphenyl was not detected in the group receiving diphenyl-labelled boscalid. Individual metabolite recoveries from urine and faeces after dosing with diphenyl-labelled boscalid and pyridyl-labelled boscalid are described in Tables 9–14.

In all dose groups and independent of sex and label, the parent substance boscalid was the major component and represented from 57% to 85% of the administered dose in the groups at the highest dose and from 30% to 41% in the groups at the lowest dose. The 4'-hydroxyl diphenyl metabolite (M510F01) and the 2-sulfydryl metabolite (M510F06) derived from the cysteine conjugate of the 2-chloro-pyridine moiety were identified to be predominant metabolites in all dose groups. The metabolites M510F20 and M510F63 were mainly found in the groups at the lowest dose. As minor metabolites, M510F05, M510F11 and M510F48 were also found in the faeces.

Major metabolites in bile were the glucuronyl conjugate of the 4'-hydroxyl diphenyl metabolite (M510F02) and M510F05 in which cysteine is conjugated with the 2-chloro-pyridine moiety. As minor components, the 4'-hydroxyl diphenyl metabolite (M510F01), and the metabolites M510F57 and M510F58 (in which there has been introduction of a hydroxyl group and cysteine into the diphenyl moiety) were identified. In addition, traces of M510F50 (hydroxyl group on the pyridine moiety) were found. The percentages of these metabolites found in bile are given in Table 15.

Table 9. Summary of metabolites identified in urine and faeces of rats given [diphenyl U-^{14}C]boscalid at a dose of 500 mg/kg bw

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F02	1.73	1.60	—	—
M510F42	0.10	0.03	—	—
M510F03	—	0.08	—	—
M510F48	0.37	0.39	1.26	1.05
M510F04	—	0.13	—	—
M510F05	0.34	0.34	0.64	0.70
M510F01	0.57	2.23	4.32	9.06
M510F20	0.02	0.02	0.80	0.57
Boscalid	—	—	80.46	64.96
M510F06	—	—	6.10	10.29

From Grosshans & Knoell (2001)

Table 10. Summary of metabolites identified in urine and faeces of rats given [pyridine 3-^{14}C]boscalid at a dose of 500 mg/kg bw

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F47	0.07	0.06	—	—
M510F02	2.74	2.14	—	—
M510F42	0.13	0.04	—	—
M510F03	—	0.13	—	—
M510F48	0.44	0.33	6.50	0.38
M510F04	—	0.10	—	—
M510F05	0.40	0.37	4.87	1.52
M510F01	0.51	1.02	8.18	10.54
M510F20	—	0.01	1.05	0.73
Boscalid	—	—	75.63	56.96
M510F06	—	—	1.04	12.24

From Grosshans & Knoell (2001)

Table 11. Summary of metabolites identified in urine and faeces of rats given [diphenyl U-^{14}C]boscalid at a dose of 50 mg/kg bw

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F02	2.95	4.33	—	—
M510F42	0.18	0.25	—	—
M510F48	1.10	2.28	—	2.84
M510F04	0.08	0.22	—	—
M510F05	0.48	0.59	—	1.85
M510F01	9.58	15.79	21.81	18.99
M510F11	—	—	2.31	0.53
M510F20	0.57	0.46	6.21	3.79
Boscalid	—	0.06	41.00	30.45
M510F06	—	—	4.88	7.57
M510F63	—	—	0.60	4.01

From Grosshans & Knoell (2001)

Table 12. Summary of metabolites identified in urine and faeces of rats given boscalid as 15 daily oral doses at 500 mg/kg bw (14 doses non-radiolabelled, last dose with radiolabelled [diphenyl U-^{14}C]boscalid)

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F02	0.22	1.03	—	—
M510F03	—	0.07	—	—
M510F42	0.26	—	—	—
M510F48	0.02	0.26	—	—
M510F04	—	0.02	—	—
M510F05	0.04	0.08	—	—
M510F01	1.34	1.94	2.46	12.60
M510F11	—	—	0.14	—
M510F12	0.06	—	—	—
M510F20	0.14	0.04	—	0.51
Boscalid	0.11	0.05	85.15	75.82
M510F06	—	—	2.60	1.41
M510F63	—	—	0.14	—

From Grosshans & Knoell (2001)

Table 13. Summary of identified metabolites in urine and faeces of rats given [diphenyl U-^{14}C]boscalid at a dose of 500 mg/kg bw[a]

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F02	0.69	2.41	—	—
M510F42	0.22	0.08	—	0.20
M510F03	—	—	—	—
M510F48	0.03	0.47	0.42	0.63
M510F04	—	0.04	—	—
M510F05	0.09	0.07	—	—
M510F01	1.04	1.52	4.10	5.50
M510F11	—	—	1.33	0.58
M510F20	0.05	0.10	—	—
Boscalid	0.16	0.04	80.37	68.26
M510F06	—	—	7.00	3.00
M510F63	—	—	0.32	1.35

From Grosshans & Knoell (2001)

[a] Dose groups from the kinetic study (see Table 1)

Table 14. Summary of identified metabolites in urine and faeces of rats given [pyridine 3-^{14}C]boscalid at a dose of 500 mg/kg bw[a]

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F47	0.10	0.07	—	—
M510F02	0.08	1.64	—	—
M510F42	0.48	0.05	—	—
M510F48	0.04	0.26	—	—
M510F04	0.01	0.01	—	—
M510F05	0.17	0.03	—	—
M510F01	2.93	0.94	4.84	4.35
M510F12	0.34	0.04	—	—
M510F20	0.26	0.06	—	—
Boscalid	0.07	0.02	72.91	70.16
M510F06	—	—	7.59	3.81
M510F63	—	—	0.46	0.25

From Grosshans & Knoell (2001)

[a] Dose groups from the kinetic study (see Table 1)

Table 15. Summary of metabolites identified in bile of female rats given [diphenyl U-^{14}C]boscalid as a single oral dose at 50 mg/kg bw or 500 mg/kg bw

Metabolite	Total excretion (% of administered dose)	
	Dose (mg/kg bw)	
	50	500
M510F02	19.27	4.78
M510F57	1.32	0.41
M510F58	0.27	0.09
M510F03	1.48	0.21
M510F22/F23	—	0.10
M510F05	14.24	3.59
M510F01	1.71	0.28

From Grosshans & Knoell (2001)

All metabolites were present in the liver at less than 0.3% of the administered dose. The metabolites M510F01, M510F02, M510F05, and M510F06 already identified in urine and faeces were also found in the liver. In addition, the metabolite M510F46 (introduction of a hydroxyl group and a glutathione group in the diphenyl ring moiety), the metabolite M510F45 (introduction of a glutathione group in the diphenyl ring moiety), and the metabolite M510F43 (exchange of chlorine for glutathione in the pyridine moiety) were identified (Table 16).

Table 16. Summary of metabolites identified in the liver of rats given [pyridine 3-^{14}C]boscalid as a single oral dose at 50 mg/kg bw or 500 mg/kg bw

Metabolite	Concentration of metabolites (µg equivalents per g)			
	Dose (mg/kg bw)			
	50		500	
	Male	Female	Male	Female
M510F47	—	—	0.21	—
M510F46	1.63	1.11	2.63	4.22
M510F02	1.88	3.08	19.43	16.42
M510F42	0.17	—	—	—
M510F45	0.66	0.41	4.04	8.25
M510F43	0.92	2.16	13.54	12.34
M510F05	0.13	0.29	2.08	1.13
M510F01	0.84	0.73	2.19	2.50
Boscalid	0.12	0.21	1.01	1.10
M510F06	0.33	0.43	2.92	3.66

From Grosshans & Knoell (2001)

The kidney revealed the metabolites M510F01, M510F02, M510F03, M510F05, M510F06, M510F48 and parent already identified in excreta. The proportion of these metabolites was in the range of <0.01% to 0.06% of the administered dose (Table 17).

Table 17. Summary of metabolites identified in kidney of rats given [pyridine 3-^{14}C]boscalid as a single oral dose at 50 mg/kg bw or 500 mg/kg bw

Metabolite	Concentration of metabolites (µg equivalents per g)			
	Dose (mg/kg bw)			
	50		500	
	Male	Female	Male	Female
M510F02	1.72	1.18	5.60	5.95
M510F42	0.20	—	—	—
M510F03	0.17	—	2.83	—
M510F48	0.30	0.80	3.00	3.23
M510F05	0.27	3.04	2.61	28.85
M510F01	0.48	0.58	2.37	3.98
Boscalid	0.68	1.69	6.92	10.48
M510F06	0.10	0.14	2.68	1.62

From Grosshans & Knoell (2001)

In plasma, M510F01, M510F02, M510F06, M510F48 and parent were detected at or less than 0.01% of the dose (Table 18).

Table 18. Summary of metabolites identified in blood plasma of rats given [pyridine 3-^{14}C]boscalid as a single oral dose at 50 mg/kg bw or 500 mg/kg bw

Metabolite	Concentration of metabolites (µg equivalents per g)			
	Dose (mg/kg bw)			
	50		500	
	Male	Female	Male	Female
M510F02	0.39	0.54	1.62	2.86
M510F48	0.12	0.07	0.91	0.52
M510F01	0.07	0.09	0.22	0.26
BAS 510F	0.26	0.29	1.74	1.85
M510F06	0.10	0.19	1.38	1.86

From Grosshans & Knoell (2001)

Metabolic pathway

After oral administration of [^{14}C]boscalid, the unchanged parent compound was predominantly found in faeces and in trace amounts in the urine, liver, and in plasma. Overall, comparison of the sexes, the different labels, and the different doses showed no remarkable differences in the metabolite patterns. The absorbed boscalid was intensively metabolized following two principal routes and a secondary route.

One principal reaction was oxidation at the 4-position of the phenyl ring, followed mainly by conjugation with glucuronic acid and to a smaller extent by sulfate. The other principal reaction was substitution of the chlorine of the 2-chloropyridine moiety by conjugation with glutathione. The glutathione moiety was then cleaved to the cysteine conjugate followed by further cleavage to the SH-compound and subsequent *S*-methylation, *S*-glucuronidation or oxidation to a sulfate.

The discovery of 2-chloronicotinic acid in urine (using the pyridine label) indicates hydrolysis of the amide bond between the pyridine ring system and the diphenyl ring system. However, the other cleavage product, chloro-aminobiphenyl, could not be detected using the diphenyl label and cleavage of the parent compound at the amide bond appeared to be negligible.

To a smaller extent, the introduction of glutathione could also occur in the diphenyl ring system followed by cleavage steps down to the SH-compound and subsequent *S*-methylation. Combinations of these reactions led to the large number of observed metabolites. The proposed metabolic pathway is shown in Figure 5. The structures of the identified metabolites are shown in Table 19 (Grosshans & Knoell, 2001).

Figure 5. Metabolic pathway of boscalid in rats

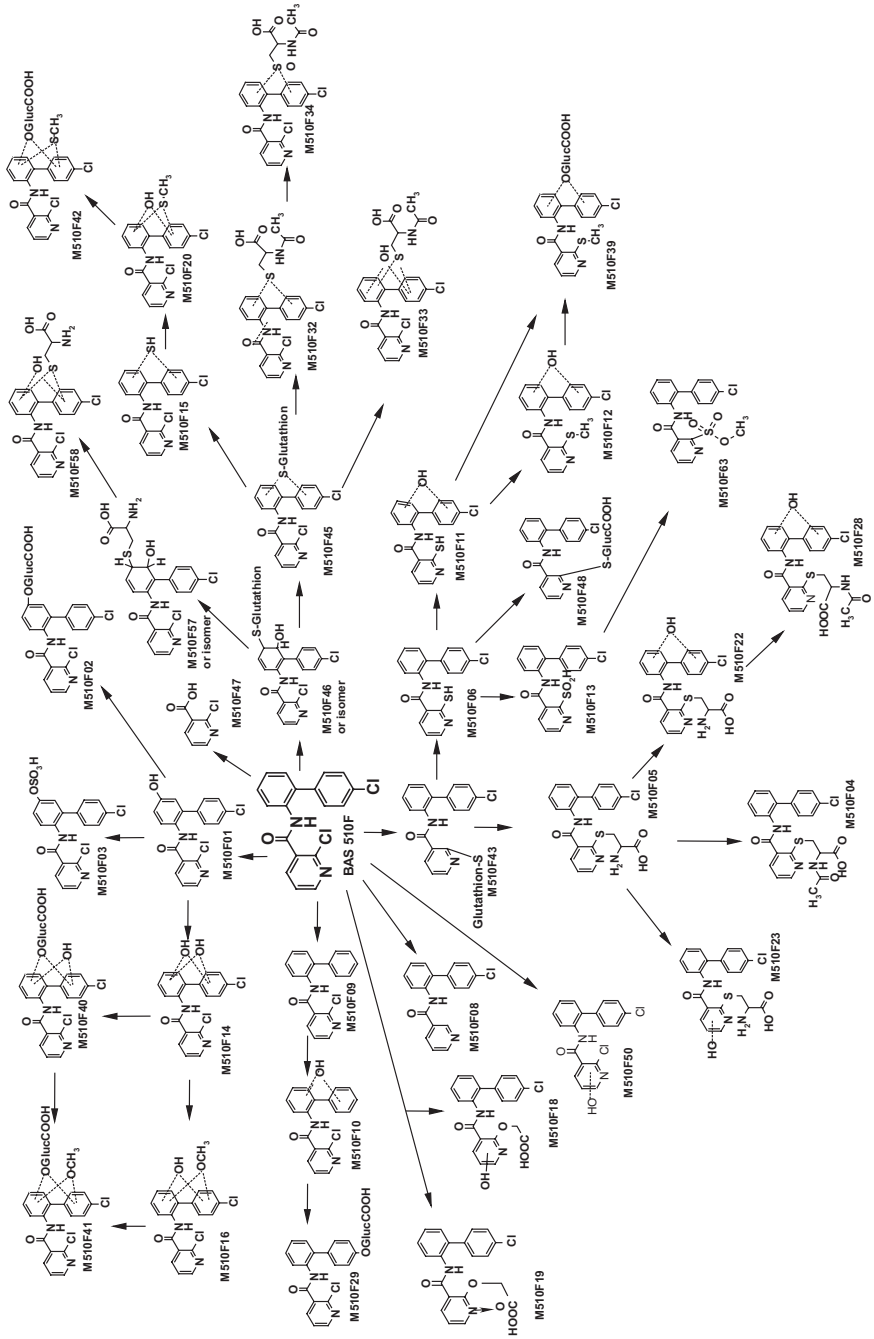
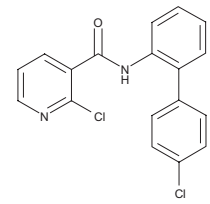
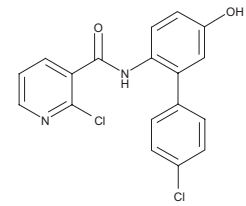
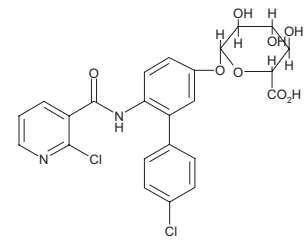
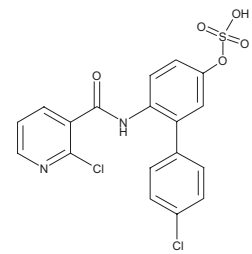
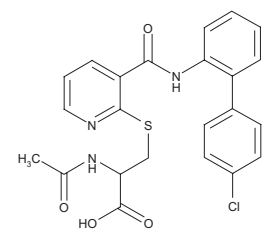
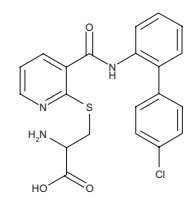
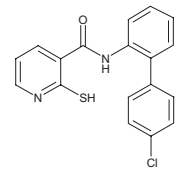
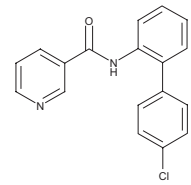
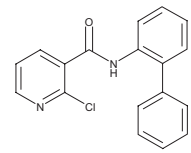
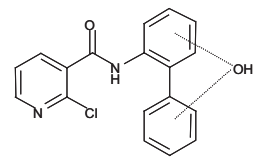
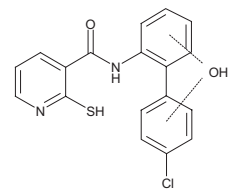
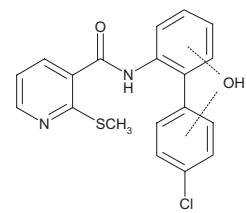
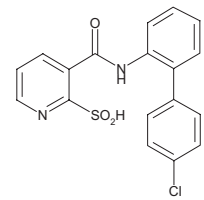


Table 19. Structures of identified metabolites in excreta, plasma and tissues of rats

Metabolite	Structure
BAS 510F	
M510F01	
M510F02	
M510F03	
M510F04	
M510F05	

Metabolite	Structure
M510F06	
M510F08	
M519F09	
M510F10	
M510F11	
M510F12	
M510F13	

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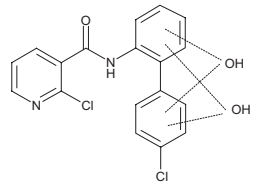
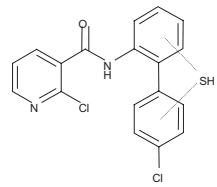
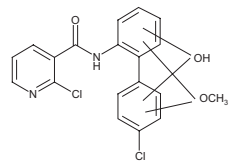
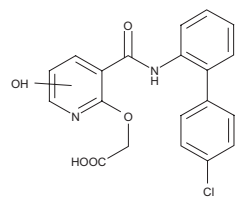
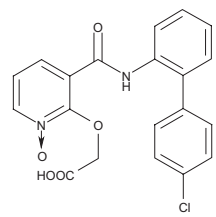
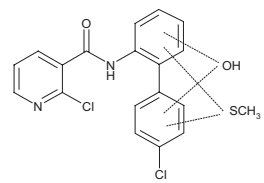
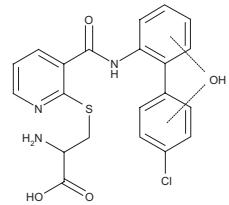
M510F14	
M510F15	
M510F16	
M510F18	
M510F19	
M510F20	
M510F22	

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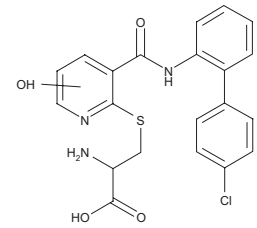
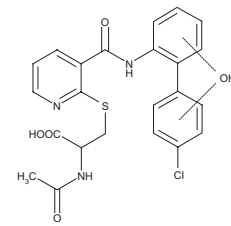
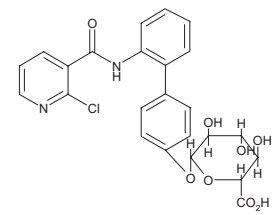
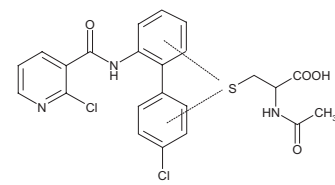
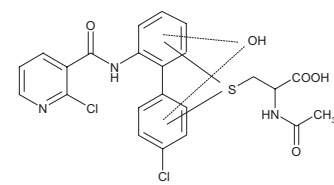
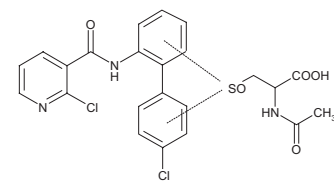
M510F23	
M510F28	
M510F29	
M510F32	
M510F33	
M510F34	

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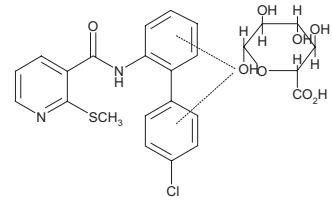
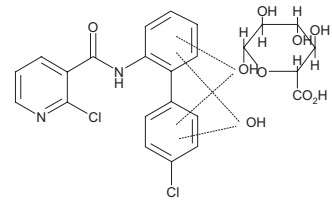
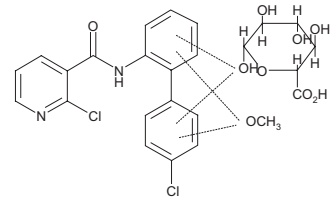
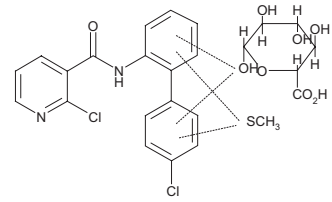
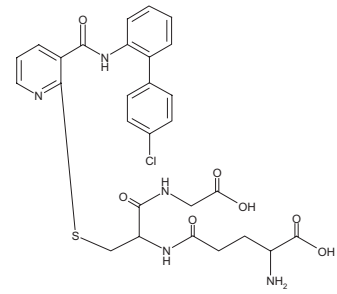
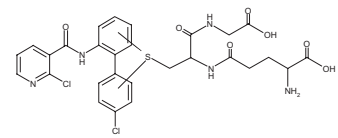
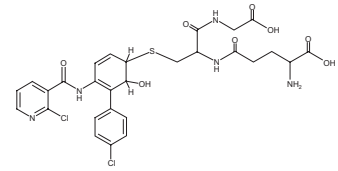
M510F39	
M510F40	
M510F41	
M510F42	
M510F43	
M510F45	
M510F46 or isomer	

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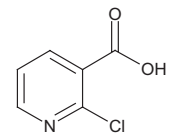
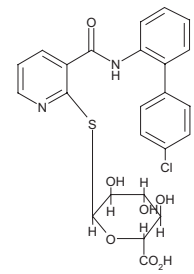
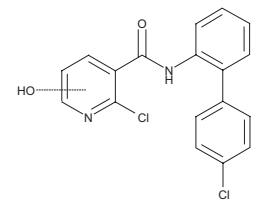
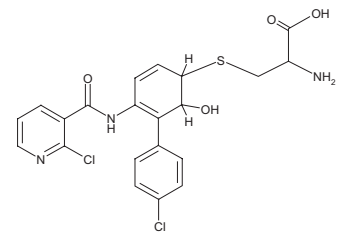
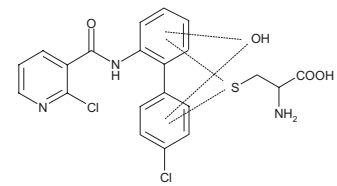
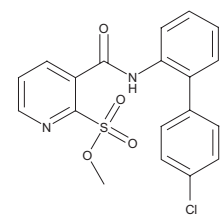
M510F47	
M510F48	
M510F50	
M510F57 or isomer	
M510F58	
M510F63	

From Grosshans & Knoell (2001)

2. Toxicological studies

2.1 Acute toxicity

The acute oral toxicity of boscalid (batch No. N 26, purity 95.3% was evaluated in groups of five male and five female fasted Wistar rats given boscalid as a single dose at 2000 or 5000 mg/kg bw by gavage in 0.5% aqueous Tylose CB 30.000, using dose volumes of 10 and 20 ml/kg bw respectively. The rats were then observed for up to 14 days. The stability of boscalid and the homogeneity and concentrations of the dosing solutions were confirmed by analysis.

There was no mortality in either males or females. Signs of toxicity noted at 5000 mg/kg bw included impaired general condition, dyspnoea, excitation, erythema and piloerection in males and females. These signs were observed on day 1 in two males and in one female. All rats appeared normal within 2 days after dosing. Body-weight development was normal. There were no macroscopic pathological findings in rats killed at the end of the observation period (Wiemann & Hellwig, 1998c; Wiemann, 2000e). The oral LD_{50} was > 5000 mg/kg bw in male and female rats.

The acute percutaneous (dermal) toxicity of boscalid (batch No. N 26, purity, 95.3%) was investigated in five male and five female Wistar rats given the test material at a dose of 2000 mg/kg bw as a 0.5% aqueous Tylose CB 30.000 preparation under a semi-occlusive dressing for 24 h. The application area was about 50 cm^2. The stability of boscalid, its homogeneity and concentration in the vehicle was confirmed by analysis.

No mortality occurred during the 14 days of observation after application.

No clinical signs of toxicity were observed and body-weight development was normal. One day after application, a well-defined erythema was observed in a single female on the first day after application. Body-weight development appeared to be normal. There were no macroscopic pathological findings in rats killed at the end of the observation period (Wiemann & Hellwig, 1998b; Wiemann, 2000a). The dermal LD_{50} for boscalid in male and female rats was > 2000 mg/kg bw.

A test for the acute toxicity of boscalid (batch No. N 26, purity 95.3%) was conducted in a group of five male and five female Wistar rats exposed by inhalation via a head/nose inhalation system for 4 h to boscalid at a mean analysed atmospheric concentration of 6700 µg/l. The observation time was 14 days. Boscalid was demonstrated to be stable and homogeneously distributed in the exposure atmospheres. Particle size analysis of the boscalid sample used revealed a mass median aerodynamic diameter (MMAD) of 3.4 µm, which is within the respirable range. No mortalities occurred in the test group. Clinical observations included attempted escape behaviour, irregular respiration and respiratory sounds, as well as urine-smeared fur, piloerection and squatting posture. No unusual clinical observations were made from day 3 after exposure onward. Body-weight development was not adversely affected by the test substance exposure. No macroscopic pathological findings were noted in exposed animals at the end of the study. The inhalation LC_{50} of boscalid in male and female rats was > 6700 mg/m^3 (Gamer & Hoffmann, 1998).

Boscalid (batch No. N 26, purity, 95.3%) was evaluated for acute dermal irritation potential in two male and four female New Zealand White rabbits. Boscalid (0.5 g) was applied to the intact skin for 4 h on a 2.5 cm × 2.5 cm test patch under a semi-occlusive dressing. After the patches were removed, the treated area was rinsed with Lutrol and Lutrol/water (1:1). The skin irritation was scored at 1, 24, 48 and 72 h after removal of the test material. The stability of the test substance over the study period was confirmed. Results from the individual animals are shown in Table 20.

Table 20. Skin irritation scores (erythema/oedema) in rabbits exposed to boscalid

Rabbit No.	Time after patch removal (h)				Mean
	1	24	48	72	
1	1/0	0/0	0/0	0/0	0/0
2	1/0	0/0	0/0	0/0	0/0
3	1/0	1/0	0/0	0/0	0.3/0
4	2/0	1/0	0/0	0/0	0.3/0
5	1/0	1/0	0/0	0/0	0.3/0
6	0/0	0/0	0/0	0/0	0/0

From Wiemann & Hellwig (1998d) and Wiemann (2000b)

The average score (24–72 h) for dermal irritation was 0.2 for erythema and 0.0 for oedema. Skin findings were reversible within 48 h in all rabbits (Wiemann, & Hellwig, 1998d; Wiemann, 2000b). The Meeting concluded that boscalid was not irritant to the skin of rabbits.

Boscalid (batch No. N 26, purity, 95.3%) was evaluated for acute eye irritation potential in two male and four female New Zealand White rabbits. Approximately 0.1 ml of undiluted boscalid was applied to the conjunctival sac of an eye of each rabbit. The test substance was washed from the eyes after 24 h. Examination of the eyes was carried out 1, 24, 48 and 72 h after the application of the test substance. The homogeneity of the test substance and its stability over the study period were confirmed. Eye irritation scores for the individual rabbits are shown in Table 21.

Table 21. Eye irritation in rabbits exposed to boscalid

Rabbit No.	Corneal opacity	Iris	Conjunctival redness	Conjunctival swelling
1	0	0	0.3	0
2	0	0	0.7	0
3	0	0	0.3	0
4	0	0	0.3	0
5	0	0	0.3	0
6	0	0	0.3	0

From Wiemann & Hellwig (1998e) and Wiemann (2000c)

The mean eye irritation scores were 0.0 for corneal and iridial effects, 0.4 for conjunctival redness and 0.0 for swelling. All effects were reversible within 72 h after application (Wiemann & Hellwig, 1998e; Wiemann, 2000c). The Meeting concluded that boscalid was not irritant to the eyes of rabbits.

The potential of boscalid (batch No. N 26, purity, 95.3%) to produce delayed contact hypersensitivity in Pirbright White (Dunkin-Hartley) guinea-pigs was tested using the Magnusson-Kligman maximization test. In a preliminary test, it was established that a concentration of 5% boscalid in 1% Tylose CB 30.000 was non-irritant, while 10% boscalid in the same vehicle was irritant. The stability of boscalid over the study period and in the vehicle was confirmed by analysis. Homogeneity of the preparation was ensured by stirring.

Twenty guinea-pigs were used in the test group and 10 in the control group. The first phase of induction was conducted by intracutaneous injections (two of each preparation per guinea-pig) of 5% boscalid in 1% Tylose CB 30.000, the same preparation with Freund adjuvant or Freund adjuvant with

1% Tylose CB 30.000. The injection sites were examined after 24 h. One week after the intradermal induction, the second phase of induction was conducted, consisting of two percutaneous applications separated by an interval of 24 h of 2 × 2 cm filter paper squares containing 25% boscalid under occlusive dressings. The challenge was performed 2 weeks after the dermal induction, by applying 5% boscalid on filter paper under occlusive dressing for 24 h. Skin reactions were scored at 24 h and 48 h after patch removal. After the intradermal application of 5% boscalid in 1% aqueous Tylose CB 30.000, there was a well defined erythema and moderate edema in all guinea-pigs.

Separate tests using α-hexylcinnamaldehyde as a positive control had been conducted twice per year in the laboratory concerned to demonstrate the continuing ability of the test procedures to detect sensitizing compounds.

One guinea-pig in the group receiving boscalid and one guinea-pig in the vehicle control group died from pneumonia unrelated to treatment. The numbers of guinea-pigs with skin reactions after the challenge are summarized in Table 22. A few animals (4 out of 19) in the group receiving boscalid showed skin reactions at challenge, while vehicle alone caused no skin irritation (Wiemann & Hellwig, 1998a; Wiemann, 2000d).

Table 22. Maximization test in guinea-pigs exposed to boscalid: results of challenge

Group	No. of positive reactions/No. of guinea-pigs tested		
	24 h	48 h	Total
Control group	0/10	0/10	0/10
Test group	3/19	4/19	4/19

From Wiemann & Hellwig (1998a) and Wiemann (2000d)

The evaluation criteria used were those of the EEC Directive 93/21 for the 18th Amendment of the Directive 67/548 EEC (Publication No. L 110A, May 4th, 1993). This requires a minimum of 30% of the test animals to show skin reactions for a classification as a sensitizer. On the basis of these criteria, the results indicated that boscalid is non-sensitizing, since only 4 out of 19 guinea-pigs (21%) showed a very slight skin reaction at challenge.

2.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female C57BL mice were given diets containing boscalid (batch No. N 26, purity, 95.3%) at a concentration of 0, 150, 1000, 4000 or 8000 ppm, equal to 0, 29, 197, 788 and 1518 mg/kg bw per day in males and 0, 42, 277, 1184 and 2209 mg/kg bw per day in females, for about 3 months. The stability of boscalid in the diet was verified and the homogeneity of the dietary mixtures was verified before the start of the study. Analyses for correct concentrations were performed before the study start and at week 8.

Food consumption and body weight were determined once per week. The state of health was checked twice per day. Blood samples were taken from all mice for haematology and blood chemistry examination at the end of the dosing period. All mice were subjected to complete gross examinations, and weights of selected organs were determined. Histopathological examinations were conducted on all organs from the control group and the group at the highest dose and on lung, liver, kidneys and all gross lesions from all dose groups.

There were no mortalities in the study and boscalid did not cause clinical signs of toxicity in any of the dose groups. There were no boscalid-related effects on body weight, food consumption

or haematology at any dose. Blood chemistry examination showed a dose-dependent, statistically significant decrease in serum cholesterol of 12%, 26% and 28%, respectively, in male mice at 1000 ppm and greater. The toxicological significance of the reduced cholesterol is not clear, since reduction of cholesterol is not associated with a pathognomonic effect, i.e. one that is indicative of a specific condition. In addition, there were statistically significant reductions in serum total protein, albumin and globulin in male mice at 4000 and 8000 ppm. Slightly increased alanine aminotransferase activities were found in serum of female mice at 4000 and 8000 ppm, but not in male mice. The reduced protein values in males and the increased values of alanine aminotransferase activities in females might indicate that liver function was impaired at higher doses.

Liver weights were significantly increased in male mice at 1000, 4000 and 8000 ppm by approximately 12%, 11% and 27%, respectively. In female mice, liver weights were significantly increased in all groups except at 1000 ppm (where the increase was not statistically significant) by approximately 8%, 7%, 12% and 22%, respectively. Liver weights relative to body weights were also increased in male and female mice at a dietary concentration of 1000 ppm and greater. The Meeting noted that the liver weights of the male and female mice of the control group were lower than those in the lowest range for control groups in the historical database, which included eight studies. Including the present study, the historical mean for males was 1208.3 mg (range, 1096.8–1443.0 mg, the first value being the value for controls in the present study). Similarly, for females the historical mean liver weight for controls was 969.7 mg (range, 886.9–1053 mg, the first value being the value for controls in the present study). There were no significant changes in weights of other organs that could be attributed to treatment.

Histopathology did not reveal any treatment-related effects in any organ except for the liver. Steatosis of varying intensity (grade 2, slight, to grade 4, marked) was recorded in all control and treated mice. This fatty change was, mostly, diffusely distributed, but with a centrilobular pronouncement. No treatment-related gain in fatty infiltration was observed in female mice, but in male mice grade 4 was recorded with dose-related incidences of 0 out of 10, 0 out of 10, 0 out of 10, 3 out of 10 and 5 out of 10, respectively.

The target organ in this study in mice was the liver, with a sex-related difference in response to treatment with boscalid. Although hypocholesterolaemia does not appear to have toxicological significance, the observation of reductions in serum concentrations of several important chemicals synthesized by the liver probably should not be ignored. The observation of increased lipid accumulation in males in the groups receiving the two higher doses would also suggest hepatic malfunction. Therefore, the Meeting considered that the liver weight changes observed at 1000 ppm and greater in both sexes were indications of an adverse effect, although this effect was more pronounced in male mice and was not accompanied by histological change until a dietary concentration of 4000 ppm. The no-observed-adverse-effect level (NOAEL) in mice given diets containing boscalid for 3 months was 150 ppm, equal to 29 mg/kg bw per day in males and 42 mg/kg bw per day in females, on the basis of increased liver weight at 1000 ppm, equal to 197 mg/kg bw in male mice and 277 mg/kg bw in female mice (Mellert et al., 2000a).

Rats

Groups of 10 male and 10 female Wistar rats were given diets containing boscalid (batch No. N 26, purity, 95.3%) at a dietary concentration of 0, 100, 500, 2000, 5000 or 15 000 ppm, equal to 0, 7, 34, 137, 347 and 1055 mg/kg bw per day in males and 0, 8, 40, 159, 395 and 1225 mg/kg bw per day in females, for about 3 months. The stability of boscalid in the diet was verified and the homogeneity of the dietary mixtures was verified before the start of the study. Analyses for correct concentration were performed before the start of the study and at week 8.

Food consumption and body weight were determined once per week. State of health was checked twice daily. Ophthalmological examinations were carried out before the start and towards

the end of dosing. Blood samples were taken from all rats for haematology and blood chemistry examination at the end of the dosing period. Urine was analysed at the end of the exposure period. All rats were subjected to complete gross examinations, and weights of selected organs were determined. Histopathological examinations were conducted on all organs from animals in the control group and in the group at the highest dose and on lung, thyroid, liver, kidneys and all gross lesions from all dosed groups.

There were no mortalities in the study and boscalid did not cause clinical signs of toxicity in any of the dosed groups. There were no boscalid-related effects on body weight or food consumption at any dose.

Erythrocyte counts and erythrocyte volume fraction values were increased in male rats at a dietary concentration of 2000 ppm and greater, and haemoglobin concentrations were increased in male rats at 5000 and 15 000 ppm. No corresponding haematological changes were observed in female rats, but prothrombin time was significantly reduced in females at 15 000 ppm.

Blood chemistry examination showed dose-dependent, statistically significant increases in gamma-glutamyltransferase activity in male rats at 2000, 5000 and 15 000 ppm and in female rats at 5000 ppm and greater. In addition, alkaline phosphatase activity was decreased in female rats at 500 ppm and greater, having significantly increased at 100 ppm. A decrease in the activity of this enzyme is generally not considered as adverse. No other enzyme activities were affected by treatment. Concentration of bilirubin decreased with increasing dose in male rats at 2000 ppm group and greater, but not in females, and the concentrations of triglycerides were reduced in males and females at 15 000 ppm. Other blood chemistry changes were increases (in contrast with mice in the previous study) in total protein and albumin in males at 5000 and 15 000 ppm and in total protein, albumin, globulin and cholesterol in females at 15 000 ppm.

There were no test-substance related effects seen in urine analysis and ophthalmoscopy.

Organ-weight measurement revealed increased weight of the liver and the thyroids in male and female rats. Liver weights were significantly increased in the group at 15 000 ppm by approximately 19% in male rats and 23% in female rats. Female rat liver weight was also increased in the group at 5000 ppm by approximately 9%. Thyroid weights were significantly increased in groups of male rats at 2000 and 15 000 ppm (but not at 5000 ppm) by approximately 21% and 34% and in groups of female rats at 5000 and 15 000 ppm by approximately 17% and 31%. Liver weights relative to body weights were also increased in male and female rats in groups at 5000 and 15 000 ppm and relative thyroid weights were increased in males at 2000 and 15 000 ppm and in females at 5000 and 15 000 ppm.

Centrilobular (zone 3) hypertrophy was recorded in 8 out of 10 male and 2 out of 10 female rats at 5000 ppm and in 10 out of 10 male and 7 out of 10 female rats at 15 000 ppm. It was also noted that in those cases where centrilobular hypertrophy occurred in male rats, fat accumulation was observed more commonly in the periphery of the lobules than in mid-zonal (zone 2) areas. All other hepatic lesions were unrelated to treatment.

In thyroids, follicular cell hypertrophy and diffuse hyperplasia of grade 1 (minimal) or 2 (slight) were recorded in some male rats of all groups. The incidences of grades 1 and 2 hypertrophy and hyperplasia were 1 out of 10, 2 out of 10, 3 out of 10, 7 out of 10, 7 out of 10 and 8 out of 10 in the groups at 0, 100, 500, 2000, 5000 and 15 000 ppm, respectively. There were no similar records of hypertrophy or hyperplasia in female rats, in spite of the organ-weight changes that had been observed. All other thyroid lesions were unrelated to treatment. Also, no lesions recorded in other organs were treatment-related. Many of the dose-related hepatic changes that were found are indicative of an adaptive response to a chemical that is an inducer of enzymes. This hypothesis was examined in another study that demonstrated a proliferation of smooth endoplasmic reticulum (see below). Such changes are fully reversible as long as degenerative or necrotic changes do not also occur in the liver. Degenerative changes were not described in this study. In the thyroid, hypertrophy and hyperplasia

incidences were increased in a dose-related fashion. However, since these observations can, and did, occur in rats in the control group, it is necessary to take note of the frequency of their occurrence.

Taking these factors into consideration, the Meeting concluded that the NOAEL in male rats given diets containing boscalid for 3 months was 500 ppm, equal to 34 mg/kg bw per day, on the basis of altered clinical-chemical changes (increases in gamma-glutamyltransferase activity) and increased incidences of thyroid hypertrophy and hyperplasia in male rats at 2000 ppm, equal to 137 mg/kg bw in male rats and this dose, equal to 159 mg/kg bw could be considered as a NOAEL in female rats (Mellert et al., 2000b).

Groups of 10 male and 10 female Wistar rats were given boscalid (batch No. N 46; purity, 96.3%) at a dose of 0 (vehicle control; 0.5% aqueous carboxymethylcellulose and 0.5% Cremophor EL solution), 100, 250 or 1000 mg/kg bw per day by the dermal route under semi-occlusive dressing, for 6 h per day, 5 days per week, for 4 weeks.

Food consumption and body weight were determined weekly. The rats were examined for signs of toxicity or mortality at least once a day. Additionally, clinical examinations were carried out before daily treatment. Detailed clinical examinations in an open field were conducted before the start of the dosing period and weekly thereafter. Ophthalmological examinations were carried out before the start and towards the end of the dosing period. Urine analysis, blood chemistry and haematological examinations were carried out at the end of the dosing period. All rats were subjected to gross pathological assessment, followed by histopathological examinations. The stability of boscalid in the vehicle was verified and the homogeneity of the dose preparations was verified. Analyses for correct concentration were performed.

One female rat receiving the highest dose died on day 13 of the dosing period. The cause of death appeared to be septicaemia. The post-mortem findings indicating cause were localized in the uterus (pyometra) and urinary tract (papillary necrosis and severe suppurative pyelonephritis of the kidney, dilation and inflammation of the urinary bladder and ureter). These changes were unrelated to boscalid administration.

There were no signs of skin irritation at any dose.

The only finding in this study was a statistically significant reduction in total bilirubin in the serum of male rats at 1000 mg/kg bw (3.02 µmol/l in controls vs 2.64 µmol/l; $p < 0.05$). The 13% reduction in serum bilirubin concentration could be related to the induction of hepatic metabolic enzymes. A similar reduction was observed in the 3-month dietary study of boscalid. Also, boscalid has been shown to increase the activity of various microsomal enzymes (see below). Therefore, these changes (in the absence of any other pathologically relevant observations) are considered to be signs of an adaptation rather than toxicologically relevant adverse effects. There were no other test-substance related changes at any dose. The NOAEL in rats exposed dermally for 4 weeks was 1000 mg/kg bw per day, the highest dose tested (Mellert et al., 2000c).

Dogs

Groups of five male and five female pure-bred beagle dogs were given diets containing boscalid (batch No. N 37, purity, 94.4%) at a dietary concentration of 0, 250, 2500 or 25 000 ppm, equal to 0, 7.6, 78.1 and 728.9 mg/kg bw for males and 0, 8.1, 81.7 and 824.8 mg/kg bw for females, for 3 months.

Measurements were made of food consumption daily and body weights weekly. The dogs were examined at least once each working day for any signs of toxicity and a check for any moribund or dead animals was made twice a day, Mondays to Fridays, and once per day at weekends and on public holidays. Blood chemistry and haematological examinations as well as urine analyses were carried out once before and on two occasions during the administration period. Ophthalmological

examinations were carried out 8 days before the beginning of the administration period and on study day 91. All dogs were subjected to gross pathological assessment, followed by histopathological examinations.

The stability of boscalid in the diet was verified. The homogeneity of the mixtures and the correctness of the concentrations were demonstrated.

There was no boscalid-related mortality or clinical signs of toxicity at any dose. The faeces in all males and females at 25 000 ppm and transiently in three males and three females at 2500 ppm were soft and light-brown discoloured.

Slight body-weight losses and retarded body-weight gains were observed during the initial phases of treatment at 25 000 ppm in both sexes. Thereafter, body-weight gain was reduced in females only. At the end of the study, females in the control group had gained 1 kg of body weight while females at 25 000 ppm had gained only 0.2 kg. At the end of the study, in males at the highest dose there was no difference in body-weight gain compared with controls. Food consumption in males at the highest dose was 96% of the total food provided vs 100% in the controls. Food efficiency in males and particularly in females at 25 000 ppm was reduced.

Ophthalmoscopy revealed no pathological changes in any dose group. Blood chemistry and haematological examinations did show some test-substance related changes. In females in the group at 25 000 ppm there was a significant, 11% reduction in erythrocyte counts and a significant, 11% reduction in haemoglobin concentration at the end of the study. Midway through the study (week 6), these parameters were also lower in dogs in the group at 25 000 ppm, but not significantly so. No other haematological changes were recorded in either sex.

With regard to blood chemistry, alkaline phosphatase activities were significantly increased by approximately threefold in males at 25 000 ppm at weeks 6 and 13. In females, alkaline phosphatase activities were significantly increased at 2500 and 25 000 ppm by almost twofold and threefold, respectively, but only at week 13. The activities of alanine aminotranferase and aspartate aminotransferase enzymes were reduced at various times in the groups at 2500 and 25 000 ppm, but reductions in these activities is not normally considered to be an adverse response. Alkaline phosphatase activity is a sensitive indicator of hepatic enzyme induction in dogs and so the observed increases, in the absence of increases in other hepatic enzymes in serum, can be interpreted as being the result of enzyme induction rather than hepatotoxicity (Keller-Rupp, 1988). In reaching this conclusion, the Meeting assumed that liver was the source of this enzyme.

Triglyceride concentrations were significantly increased after 6 weeks of treatment at 25 000 ppm by approximately 2.1-fold in males and 1.8-fold in females. After 13 weeks, triglyceride concentrations were significantly increased in males at 250 ppm and greater and in females at 2500 ppm and 25 000 ppm. The increases were dose-related and reached approximately twofold in both males and females at 25 000 ppm. Although the concentration of triglycerides at 13 weeks was significantly higher in males at 250 ppm, the difference (30%) was no greater than in females (30%) and could probably be viewed as an lowest-observed-adverse-effect level (LOAEL). However, additional considerations included the fact that: (1) the control group concentrations appeared to be particularly low in this study; and (2) apart from the group values for male and females at 25 000 ppm for both sexes and for males at 2500 ppm at 6 weeks, all triglyceride concentrations were within the range for historical controls (historical data were supplied in the report for the performing laboratory for February 1995–January 1998: male beagles, 3-month studies: mean, 0.38; range, 0.24–0.45, n = 10; and female beagles, 3-month studies: mean, 0.45; range, 0.38–0.51, n = 9). On the other hand, assuming that there had been proper randomization, low concentrations of triglyceride should be a characteristic of all these dogs and the dose-related concentration increases were monotonic throughout the dose range in males and females at weeks 6 and 13. Furthermore, as in the great majority of studies in dogs, the group sizes are small and therefore totally reliance on statistically significant differences in pair-wise comparisons cannot be made.

The mean liver weights were significantly increased in the groups at 2500 and 25 000 ppm by approximately 18% and 42%, respectively, in males and by approximately 19% and 49%, respectively, in females. Liver weights relative to body weights were also significantly increased in females in these two groups and in males at 25 000 ppm. Although there were no significant increases in absolute thyroid weights, there was a trend, particularly in females, and thyroid weight relative to body weight was significantly increased in females at 25 000 ppm by approximately 43%.

No test-substance related gross or histopathological changes were observed at any dose, therefore, with the exception of the reduction in erythrocytes and haemoglobin concentration, virtually all other changes were indicative of enzyme induction rather than frank toxicity.

The NOAEL for beagle dogs was 250 ppm, equal to 7.6 mg/kg bw per day in males and 8.1 mg/kg bw per day in females, on the basis of increased serum triglyceride concentrations and effects on organ weight of liver in males and females at 2500 ppm, equal to78.1 mg/kg bw per day in males and 81.7 mg/kg bw per day in females (Schilling et al., 2000).

Groups of five male and five female pure-bred beagle dogs were given diets containing boscalid (batch No. N 37, purity, 94.4%) at a concentration of 0, 200, 800, 2000 or 20 000 ppm, equal to 0, 5.5, 21.8, 57.4 and 544.0 mg/kg bw for males and 0, 5.8, 22.1, 58.3 and 592.9 mg/kg bw for females, for about 12 months.

Measurements were made of food consumption daily and body weights weekly. The dogs were examined at least once each working day for any signs of toxicity and a check for any moribund or dead animals was made twice a day, Mondays to Fridays, and once a day at weekends and on public holidays. Blood chemistry and haematological examinations as well as urine analyses were carried out once before and after approximately 3, 6 and 12 months. Ophthalmological examinations were carried out 7 days before the beginning of the administration period and on study day 359. All dogs were subjected to gross pathological assessment, followed by histopathological examinations.

The stability of boscalid in the diet was verified. The homogeneity of the mixtures and the correctness of the concentrations were demonstrated.

There was no mortality during the study. Clinical signs of toxicity consisted of vomiting during the later part of the study in a single female at 20 000 ppm. The faeces in all males and females at 20 000 ppm were soft and light-brown discoloured.

Body weights of females in the group at 20 000 ppm initially decreased and their body-weight gain was reduced for the rest of the study period, so that the terminal mean body weights were 13.5 kg in the controls and 12.1 kg in the group at 20 000 ppm. Body-weight gain was also reduced in females at 2000 ppm, to 12.0 kg at the end of the study.

There were no treatment-related changes in the haematological parameters measured. Blood chemistry analysis showed that there were increases in alkaline phosphatase activity in male dogs at 2000 and 20 000 ppm throughout the study, although at termination the higher mean value for the males at 2000 ppm was not significantly different from that of the controls. Alkaline phosphatase activity was also increased in females in the group at 20 000 ppm throughout the study. Of the other serum enzymes measured, alanine aminotransferase activity was reduced in males at 2000 and 20 000 ppm throughout the study and alanine aminotranferase and aspartate aminotransferase activities were reduced in females at 20 000 ppm at 3 months. Decreases in the activity of these aminotransferases are not normally considered to be adverse responses.

There were significant increases in serum total protein, globulin, cholesterol and triglyceride concentrations and a decrease in chloride concentration in females at 20 000 ppm at 3 months; total protein concentrations were also increased in females at this dose at 6 months. There were no significant or dose-related increases in these parameters at 12 months. In male dogs, serum triglyceride

concentrations were increased at 20 000 ppm throughout the study and chloride concentrations were reduced at 3 months in the same group. No treatment-related changes were observed in urine.

Mean liver weights were significantly increased in female dogs at 20 000 ppm by 42% and, although the difference was not statistically significant, the liver weight in male dogs in the group at 20 000 ppm was 30% higher than that in the controls. Thyroid weights were significantly increased in males in the groups at 2000 and 20 000 ppm by approximately 39% and 54%, respectively, and were non-significantly higher in females at 20 000 ppm by approximately 42%. The weights of liver and thyroid relative to body weight were also increased in these groups of male and female dogs. The histopathological changes recorded were incidental or spontaneous and unrelated to treatment.

The NOAEL was 800 ppm, equal to 21.8 mg/kg bw per day in males and 22.1 mg/kg bw per day in females, on the basis of body-weight reductions in females and thyroid-weight increases in male and female dogs at 2000 ppm, equal to 57.4 mg/kg bw per day in males and 58.3 mg/kg bw per day in females (Wiemann et al., 2000).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female C57BL mice were given diets containing boscalid (batch No. N 37; purity, 94.4%) at a concentration of 0, 80, 400, 2000 or 8000 ppm, equal to 0, 13, 65, 331 and 1345 mg/kg bw in males and 0, 18, 90, 443 and 1804 mg/kg bw in females for 18 months.

Food consumption and body weight were determined once per week during the first 13 weeks and at 4-week intervals thereafter. A check of the general state of health of the mice was made at least daily. Additionally, the mice were examined in detail and palpated once per week. Blood smears were prepared after 12 months and 18 months, and from all mice killed in extremis. After 18 months of treatment, the mice were subjected to gross pathological assessment, measurement of organ weights and histopathology.

The stability and homogeneity of the test substance in the diet, and the correct concentrations were confirmed by analysis. There was no increase in mortality or clinical signs of toxicity in this study that could be attributed to treatment. Food consumption was not adversely affected but body weight was reduced in groups of male mice at a dietary concentration of 400 ppm and greater and in females at 8000 ppm. Although there was a monotonic reduction in mean body weight throughout the dose range for male mice, the maximum reduction was only approximately 9%.

Blood smears did not indicate any test-substance related effects.

Liver weights were increased in males at 8000 ppm by 16% and in females at 2000 and 8000 ppm by 8% and 10%, respectively. Liver weights relative to body weight also increased in males at 400 ppm and greater and in females at 2000 and 8000 ppm. Organ weights of adrenals and relative testes weights were also increased in treated groups, but these differences were either a consequence of extremely low adrenal weights in the control group (all adrenal weights being within the range for historical controls) or, in the case of testicular weights, failed to show a clear dose–response relationship. In neither case was the weight change accompanied by histological aberrations.

Histopathological investigations demonstrated an increased incidence of peripheral hypertrophy of hepatocytes in male mice, from 0 out of 50 in all groups up to and including the group at 2000 ppm to 29 out of 50 in the group at 8000 ppm, and in female mice from 0 out of 50 in all groups up to and including the group at 400 ppm to 10 out of 50 in the group at 2000 ppm and 45 out of 50 in the group at 8000 ppm. Fatty infiltration was common in all groups of males and females. In males the distribution was almost entirely centrilobular, and the more prevalent severity gradings (grades 3 and 4) were similar in all groups. In addition, low incidences of grade 5

(the highest score possible) were observed in the groups at 2000 and 8000 ppm. In females, fatty infiltration was predominantly diffuse, but shifted towards a centrilobular distribution at higher doses; however, the incidences of grades 3 and 4 severity (there was none at grade 5, the highest possible score) for centrilobular and diffuse fatty infiltration were similar in all groups.

There was no evidence for any treatment-related increased incidence of benign or malignant neoplasms in any organs of male or female mice. The Meeting concluded that boscalid is not carcinogenic in mice. The NOAEL in this 18-month study of toxicity and carcinogenicity in mice was 80 ppm, equal to 13 mg/kg bw per day in males, on the basis of reduced body-weight gain in male mice at 400 ppm, equal to 65 mg/kg bw per day. The NOAEL in female mice was 400 ppm, equal to 90 mg/kg bw per day, on the basis of liver-weight increases and hepatic peripheral hypertrophy at 2000 ppm, equal to 443 mg/kg bw per day (Mellert et al., 2001d).

Rats

Groups of 20 male and 20 female Wistar rats were given boscalid (batch No. N 37; purity, 94.4%) at a concentration of 0, 100, 500 or 2500 ppm, equal to 0, 4.4, 21.9 and 110.0 mg/kg bw per day in males and 0, 5.9, 30.0 and 150.3 mg/kg bw per day in females for 24 months. The study had begun with a dose at 15 000 ppm, equal to 739.0 mg/kg bw in males and 1000.4 mg/kg bw in females, but this was discontinued after approximately 17 months of treatment.

Food consumption and body weight were determined once per week during the first 13 weeks and at 4-week intervals thereafter. The rats were examined for signs of toxicity or mortality at least once per day; moreover, comprehensive clinical examinations and palpations of the rats were performed once per week. Ophthalmological examinations were carried out before the start and towards the end of dosing for rats in the control group and for rats at the highest dose. Blood samples were taken from all rats for haematology and blood chemistry examination after about 3, 6, 12, 13, 18 and 24 months of treatment. Urine samples were collected for analysis at about these same times. All rats were subjected to complete gross examinations, and weights of selected organs were determined. Histopathological examinations were conducted on all organs from rats at 0 and 2500 ppm and on all gross lesions, thyroids, lungs, liver and kidneys from rats at 100 and 500 ppm.

The stability of the test substance, the homogeneous distribution, stability and correct concentration of the test substance in the diet were confirmed by analysis.

The group at 15 000 ppm was discontinued after approximately 17 months because of severe effects on body weight that were expected to progress. However, this was clearly a decision taken late in the study. In comparison with the group at 0 ppm, the body-weight deficit was 8.5% in males and 14.4% in females at this stage of the study. Mortality was not affected (there being a single death in each of the groups of males and females at this dose, similar mortalities occurred in males at 100 and 2500 ppm and in females at 500 ppm at 17 months) and the blood chemistry and haematology observations were similar to those for the group at 2500 ppm (some observations being clearly adverse while several others were not).

In the remaining groups, mortality at 24 months was unaffected by treatment. Survival percentages in the groups at 0, 100, 500 and 2500 ppm, respectively, were 75%, 80%, 70% and 70% in males and 65%, 85%, 65% and 95% in females. No boscalid-related clinical observations were made and there were no significant effects on body-weight gain. There were sporadic increases in feed consumption in groups of females at 500 and 2500 ppm, but these were assessed as being incidental and not related to treatment.

No boscalid-related ophthalmic effects were observed. Decreases were observed in erythrocyte volume fraction, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) in female rats at 2500 ppm, but there were no changes in reticulocyte counts for either sex. Prothrombin

times were slightly, but significantly reduced at 3 months in females at 500 and 2500 ppm (28.8 s vs 27.6 and 26.9 s, respectively).

Serum concentrations of cholesterol were increased in male rats at 2500 ppm at 12 months and females at 3, 12, 13 and 18 months. In addition, cholesterol concentrations were increased in females at 500 ppm, at a single sampling time of 13 months (which happened to be about the same time as there was a significant increase in gamma-glutamyltransferase activity in males at this dose). Serum concentrations of bilirubin were decreased in males at 2500 ppm at almost every blood sampling time and at 500 ppm at 12 and 18 months. Reduced serum concentrations of bilirubin were found in female rats at 100 ppm and greater at 3 and 13 months and females at 2500 ppm at 24 months.

There were treatment-related changes in the activities of several serum enzymes in the study. Serum gamma-glutamyltransferase activities were increased in male rats at 2500 ppm at all blood sampling times, at 500 ppm at 3, 6 and 12 months and at 100 ppm at 3 months. The activity of this enzyme was also increased in females at 2500 ppm at 3 and 12 months. For example, the mean activities of gamma-glutamyltransferase in male rats at 12 months were (± standard deviation):14.7 ± 7, 15 ± 9, 20 ± 7** and 34 ± 20** units in the groups at 0, 100, 500 and 2500 ppm groups, respectively (** $p < 0.02$). Reduced activity was observed in serum alkaline phosphatase in male rats at 2500 ppm at all blood sampling times and at 500 ppm at 3 and 6 months. Alkaline phosphatase activity was also reduced in female rats at 2500 ppm at 3, 6, 12, 13 and 18 months and at 500 ppm at 3 months. Alanine aminotransferase activity was reduced in males at 2500 ppm at 6 and 18 months, in females of the same group at 3, 6, 12 and 13 months and at 100 and 500 ppm at 3 and 6 months. Asparate aminotransferase activity was also reduced in female rats at 2500 ppm, but only at 6 months. Although statistically significant, many of these changes were small and reductions in the activities of aminotransferases are not associated with toxicity. There were no treatment-related changes in the urine parameters examined.

The only significant changes in absolute organ weights were an approximately 31% increase in thyroid weight in male rats at 2500 ppm and an approximately 3% increase in brain weight in females at 100 ppm. The latter was clearly not treatment-related. Liver weight relative to body weight was increased by approximately 11% in females at 2500 ppm, but not in males. At autopsy, the number of male rats with enlarged thyroids or with a focus in the thyroids was slightly higher in the group at 2500 ppm. There were also a number of rats with cystic degeneration of the testes. These occurred in all groups, at incidences of 2 or 3 out of 20, except for the group at 2500 ppm, where there were 9 out of 20.

Microscopic examination revealed slightly increased incidences of diffuse hypertrophy and of focal hyperplasia of the thyroid follicular cells. A few thyroid follicular cell adenomas were also found (Table 23). In liver, there were increased incidences of centrilobular hypertrophy in males at 2500 ppm (0 ppm, 0 out of 20; 2500 ppm, 6 out of 20) and female rats (0 ppm, 0 out of 20; 2500 ppm, 16 out of 20). The incidences of eosinophilic foci were increased in males at 500 and 2500 ppm (3 out of 20, 4 out of 20, 7 out of 20 and 9 out of 20 in the groups at 0, 100, 500 and 2500 ppm, respectively), but not in females (1 out of 20, 1 out of 20, 2 out of 20 and 2 out of 20). In the testes, there was an increased incidence in cystic degeneration in the group at 2500 ppm.

Table 23. Incidences of thyroid follicular cell lesions in rats fed diets containing boscalid for 24 months

Lesion	Dietary concentration (ppm)							
	Males				Females			
	0	100	500	2500	0	100	500	2500
No. of rats in group	20	20	20	20	20	20	20	20

Diffuse hypertrophy	3	1	3	6	0	1	0	4
Focal hyperplasia	1	1	2	4	0	0	0	2
Adenoma	0	0	2	1	0	0	1	0

From Mellert et al. (2001b)

The NOAEL was 100 ppm, equal to 4.4 mg/kg bw per day in males and 5.9 mg/kg bw in females, on the basis of increased serum gamma-glutamyltransferase activity in males at 500 ppm, equal to 21.9 mg/kg bw per day in males and 30.0 mg/kg bw per day in females (Mellert et al., 2001b).

Groups of 50 male and 50 female Wistar rats were given diets containing boscalid (batch No. N 37, purity, 94.4%) at a concentration of 0, 100, 500, 2500 or 15 000 ppm, equal to 0, 4.6, 23.0, 116.1 and 768.8 mg/kg bw per day in males and 0, 6.0, 29.7, 155.6 and 1024.4 mg/kg bw per day in females for 24 months. The group at 15 000 ppm was discontinued after approximately 17 months of treatment. The conduct of this experiment overlapped the 24-month study of toxicity described above. The studies should be looked upon as largely independent of each other.

Food consumption and body weight were determined once per week during the first 13 weeks and at 4-week intervals thereafter. The rats were examined for signs of toxicity or mortality at least once per day; moreover, comprehensive clinical examinations and palpations of the rats were performed once per week. Blood smears were taken from the groups at 0 and 2500 ppm at the end of the study. All rats were subjected to complete gross examinations, and weights of selected organs were determined. Histopathological examinations were conducted on all organs from rats at 0 and 2500 ppm and on all gross lesions, thyroids, lungs, liver and kidneys from rats at 100 and 500 ppm.

The stability of the test substance, the homogeneous distribution, stability and correct concentration of the test substance in the diet were confirmed by analysis.

The group at 15 000 ppm was discontinued after approximately 17 months of treatment. In this group, there had been one death of a male rat by 14 months and this had increased to four deaths when the group was withdrawn at 17.5 months. However, there had been no deaths in females up to that time. Furthermore, male rats in the group at 15 000 ppm were gaining weight at a rate similar to the group at 0 ppm up to the time of withdrawal, although female rats at 15 000 ppm were gaining weight less rapidly than the other groups of female rats. They did not appear to have been subjected to pathological examination. The remainder of this description refers only to the groups at 0, 100, 500 and 2500 ppm.

Body weight at the end of the study was significantly reduced by 16.9% in females at 2500 ppm, but there was no similar effect in males. Food consumption was not affected by treatment. Examination of the blood smears at terminationdid not reveal any treatment-related changes in the leukocyte differential count or erythrocyte morphology.

Organ-weight changes included significant reductions in absolute kidney weight of 4.8% and 7.5% in female rats in groups at 500 and 2500 ppm, respectively. In males, there were increases in the absolute weights of the testes by approximately 20% in the group at 500 ppm only and increases in the absolute weights of the thyroids by approximately 18% in the group at 2500 ppm. In this same group, thyroid weight relative to body weight was increased by approximately 17%. At autopsy, enlarged thyroids were noted in 2 out of 50 and 1 out of 50 male rats at 500 and 2500 ppm, respectively. All other gross lesions were either single occurrences or were approximately equally distributed between the groups, including the control group.

Microscopic examination revealed an increased incidence of diffuse hypertrophy of the thyroid follicular cells in male rats at 2500 ppm (Table 22) and focal hyperplasia of the thyroid follicular cells

in males and females rats at 2500 ppm. Diffuse follicular cell hypertrophy can be viewed as indicative of increased function induced by thyroid-stimulating hormone (TSH), while the focal hyperplasia indicated continued stimulation of the thyroid to which the pre-existing cell numbers were unable to respond further. A few thyroid follicular cell adenomas were also found (Table 24). The incidence in males at 2500 ppm is considered to be almost significant (p = 0.06) according to Fisher's (one-sided) exact test. The numerical increase of thyroid follicular cell adenomas in males and females at 2500 ppm is considered to be a test-substance related effect based on the additional non-neoplastic changes in this organ. Using the United States Environment Protection Agency (EPA) benchmark dose software version 1.3.2 to fit the data to a number of statistical models, the benchmark dose lower 95% confidence limit (BMDL) was 76 mg/kg bw per day

Table 24. Thyroid follicular cell lesions in rats fed diets containing boscalid for 24 months

Lesion	Dietary concentration (ppm)							
	Males				Females			
	0	100	500	2500	0	100	500	2500
No. of rats	50	50	50	50	50	50	50	50
Diffuse hypertrophy	2	5	6	22	2	0	0	4
Focal hyperplasia	1	1	1	9	2	2	1	7
Adenoma	0	0	1	4	0	1	0	3

From Mellert et al. (2001a)

In liver, there were increased incidences of centrilobular hypertrophy in male rats in the groups at 500 and 2500 ppm (0 ppm, 0 out of 50; 100 ppm, 0 out of 50; 500 ppm, 2 out of 50; and 2500 ppm, 27 out of 50) and at 2500 ppm in female rats (0 ppm, 0 out of 50; 100 ppm, 0 out of 50; 500 ppm, 0 out of 50; and 2500 ppm, 11 out of 50). Eosinophilic foci were also recorded in the liver of male rats in all groups, but with slightly higher incidences at 500 and 2500 ppm, (8 out of 50 and 9 out of 50, respectively) compared with 3 out of 50 and 4 out of 50 at 0 and 100 ppm, respectively. No eosinophilic foci were recorded for livers of females in any group. The observed changes in the liver suggested an increased functional activity of this organ and, as will be described later, they may be the primary cause of the neoplastic response in the thyroid.

The only other proliferative response noted that appeared to be related to treatment with boscalid was a diffuse hyperplasia of the papillary transitional cells in the urinary bladder. This hyperplasia was recorded in all groups of male rats (with incidences of either 6 or 7 out of 50), but the incidence was highest (1 out of 50) in the group at 2500 ppm. The hyperplasia was accompanied by inflammation in the urinary bladder.

The NOAEL was 100 ppm, equal to 4.4 mg/kg bw per day, on the basis of increased gamma-glutamyltransferase activity and hepatic centrilobular hypertrophy and eosinophilic foci in males and increased cholesterol in females at 500 ppm, equal to 23.0 mg/kg bw per day in males and 29.7 mg/kg bw in females (Mellert et al., 2001a).

Boscalid has a tumorigenic effect in rats that is not considered to be relevant for humans, because: very high exposure levels would be required to produce such a response; the effect has a low potency, as indicated by the marginal, non-statistically significant increase in (thyroid follicular cell) adenomas and the absence of any carcinomas in rats; and the fact that rats are known to be particularly sensitive to this type of tumour compared with human beings.

Because no significant increase in the incidence of thyroid tumours was observed and all the tumours reported were benign, the Meeting considered that was not necessary to state a formal

mode of (carcinogenic) action. Nevertheless, data were available that supported the proposal that the thyroid effects are unlikely to occur in human beings. These data are summarized here.

The elements of a proposed mode of action would be that perturbation of the hypothalamus–pituitary–thyroid homeostasis beyond normal bounds leads to thyroid follicular cell hyperplasia stimulated by excess TSH. Evidence for the endocrinological changes is provided in the section on *Liver and thyroid effects*, below, where experiments are described in which rats were fed diets containing boscalid at a concentration of 15 000 ppm for 2 weeks. This evidence indicated that boscalid induces proliferation of hepatocellular smooth endoplasmic reticulum, cytochrome P450 enzymes and all of the three glucuronyltransferase enzymes examined. It is a reasonable prediction that glucuronyltransferase enzymes involved in the metabolism (conjugation) of thyroxin (T4) and triiodothyronine (T3) would also be induced, thereby accelerating their excretion. The experiment provides evidence for reductions in circulating T3 and T4. The feedback response of the hypothalamus–pituitary system to this reduction would be synthesis and release of larger quantities of TSH. Increased serum TSH was observed in the experiment. The expected consequential hyperplasia in the thyroid was observed in the long-term experiment in rats described above. All these events could occur in human beings in whom, however, the homeostatic control mechanisms are far more robust than in rats (Capen et al., 1999). Thus, the high doses required in rats to produce even a marginal increase in thyroid follicular cell adenomas would be expected to be much higher in human beings. Although many of the necessary steps for thyroid follicular cell adenoma induction by this hormonal mechanism have been demonstrated, weaknesses in the scheme are that the endocrinological data are derived from a single experiment and the single dose of boscalid investigated was well in excess of the dose used to demonstrate thyroid follicular cell hyperplasia and benign neoplasia.

2.4 Genotoxicity

Boscalid was tested for genotoxicity in a range of assays in vitro and in vivo (Table 25). There was no evidence for induction of gene mutation induction in *Salmonella typhimurium* or *Escherichi coli* (Engelhardt & Hoffman, 1998) in the standard plate assay or in a preincubation assay in vitro. The dose range used extended into a range that was bacteriotoxic for this compound. No significant response was observed in a single study of mutations at the *Hprt* locus in Chinese hamster ovary (CHO) cells in vitro, after exposure for 4 h to boscalid at concentrations up to 100 µg/ml or 1050 µg/ml in the absence or presence of an exogenous metabolic activation system, respectively (Engelhardt & Hoffmann, 2000a). Thus, boscalid did not induce gene mutations in bacterial cells or cultured mammalian cells.

In a single study on the induction of chromosomal aberrations in cultures of V79 cells, no significant increases in the proportion of abnormal cells were observed after exposure for 4 h in the presence of an exogenous activation mixture or 18 h and 28 h in its absence with boscalid at concentrations of up to 500 or 125 µg/ml, respectively (Engelhardt & Hoffmann, 1999a).

No genotoxic activity was observed in a study on the induction of unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes exposed for 18–20 h to boscalid at concentrations of up to 50 µg/ml. At a concentration of ≥ 50 µg/ml, boscalid precipitated in the culture medium and at 100 µg/ml was toxic. Treatment with 2-acetylaminofluorene at 1 µg/ml induced a substantial response under the same culture conditions (Engelhardt & Hoffmann, 2000b).

In a test for clastogenicity and aneugenicity, induction of micronucleus formation was examined in bone-marrow cells of NMRI mice. Groups of five male mice were dosed by intraperitoneal

End-point	Test object	Dose[a](LED/HID)	Purity (%)	Result	Reference
In vitro					
Gene mutation	*S. typhimurium* strains TA100, TA1535, TA1537, TA98; *E.coli* WP2*uvrA*, standard plate and pre-incubation protocols	2750 µg/plate (toxic)	95.3	Negative ± S9	Engelhardt & Hoffmann (1998)
Gene mutation	Chinese hamster ovary (CHO) cells, *Hprt* locus	500 µg/ml −S9 (precipitation at 31 µg/ml), 4 h 1000 µg/ml +S9, 4 h	94.4	Negative ± S9	Engelhardt & Hoffmann (2000a)
Chromosomal aberration	Chinese hamster lung V79 cells	500 µg/ml −S9 (precipitation at 31 µg/ml), 4 h500 µg/ml +S9 4 h; 125 µg/ml +S9, 28 h	94.4	Negative ± S9	Engelhardt & Hoffmann (1999a)
Unscheduled DNA synthesis	Liver cells from male Wistar rats	50 µg/ml, 18–20 h	94.4	Negative	Engelhardt & Hoffmann (2000a)
Micronucleus formation	Male & female NMRI mouse bone-marrow cells, 2 h after dosing	2000 mg/kg bw, intraperitoneal injection × 2	94.4	Negative	Engelhardt & Hoffmann (1999b)

[a] LED, lowest effective dose; HID, highest ineffective dose; S9, 9000 × *g* supernatant from livers of male Sprague-Dawley rats

injection with boscalid at a dose of 0, 500, 1000 or 2000 mg/kg bw on two occasions separated by 24 h. The highest dose was selected on the basis of a preliminary limit test in which two intraperitoneal injections at 2000 mg/kg bw were administered at an interval of 24 h to male and female mice. Clinical signs recorded were squatting posture, piloerection and a general poor condition that was similar in males and females. For this reason, the test for genotoxicity was conducted with a single sex (males) only. In addition, two groups of five mice were dosed with the positive-control substances cyclophosphamide (for clastogenicity) or vincristine (for aneugenicity) by intraperitoneal injection on a single occasion. All mice were killed 24 h after dosing. Target concentrations of boscalid were verified analytically. No increases in micronucleated polychromatic erythrocytes were observed in any group dosed with boscalid. Large increases in the incidence of micronuclei were observed in both of the positive-control groups (Engelhardt & Hoffmann, 1999b).

The Meeting concluded that boscalid is unlikely to be genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 25 male and 25 female sexually immature Wistar rats were fed diets containing boscalid (batch No. N 37, purity, 94.4%) at a concentration of 0, 100, 1000 or 10 000 ppm, equal to 0, 10.1, 101.2 and 1034.5 mg/kg bw per day for males and 10.7, 106.8 and 1062.0 mg/kg bw per day for females averaged over the pre-mating period of exposure of the F_0 parental rats in a two-generation study of reproduction. After 10 weeks, the rats were mated and allowed to rear the ensuing F_1 litters to weaning.

F_0 females received the same dietary concentrations during gestation and lactation, which were equal to 0, 8.7, 88.7 and 907.4 mg/kg bw per day and 0, 14.8, 149.4 and 1456.7 mg/kg bw per day, respectively. The breeding programme was repeated with the F_1 parents (selected from the F_1 offspring) after an exposure period of at least 10 weeks. The ensuing F_2 litters were reared to weaning. The diets containing boscalid were fed continuously throughout the study. The stability and homogeneous distribution of boscalid in the diet were evaluated before the beginning of the study. Analyses to confirm correct concentrations were performed periodically during the study.

Body weights of F_0 and F_1 parents were measured once per week during the pre-mating period, after which F_0 and F_1 females were weighed on days 0, 7, 14 and 20 of gestation and on postnatal days 1, 4, 7, 14 and 21. Data on the estrous cycle were evaluated for F_0 and F_1 rats during the 3 weeks before mating until evidence of mating was obtained. Sperm parameters (motility, sperm head count and morphology) were assessed in all F_0 and F_1 rats at about the time of scheduled sacrifice. The F_1 and F_2 pups were weighed on the day of or day after birth, and on postnatal days 4, 7, 14 and 21. The state of health of the parents and the pups was checked at least once per day, and parental animals were examined for their mating and reproductive performances. Pups were sexed and evaluated as to health, and pup viability was recorded. A gross pathological examination was carried out on all parents and offspring. Histopathology was conducted on all F_0 and F_1 parents, special attention being paid to the reproductive organs.

The stability and homogeneity of the test substance in the diet and target dietary concentrations were confirmed by analysis

In the parental groups (F_0 and F_1 generations), boscalid had no adverse effects on survival and there was no evidence for treatment-related clinical changes. Food consumption among the F_0

parental rats was not markedly affected by treatment over the entire treatment period, while the food consumption of F_1 parental females was clearly reduced (by about 8%) in the group at 10 000 ppm during lactation. During the pre-mating period, male and female body-weight differences between groups were small, but differences began to increase in females during gestation. During lactation, administration of diet containing boscalid at 1000 or 10 000 ppm was associated with lower body weights and body-weight gains in F_0 females. There was a similar trend, although not so obvious and not statistically significant, in the F_1 females. In the F_1 generation, mean body weights of male rats at 10 000 ppm were significantly reduced for almost the entire treatment period. The Meeting considered that this response in male rats was related to treatment.

There was no evidence for an adverse effect of boscalid on sperm parameters, mean coital time, fertility index, conception rate, duration of gestation, or gestation index at any of the doses tested. The parental females showed regular estrous cycles over the 3-week observation period before mating and became sperm-positive within a few days after mating. There was no effect on litter size at birth that could be attributed to boscalid. Pup viability was unaffected by treatment in the F_1 generation, but there mortality of F_2 generation pups at age 0–4 days was higher at 10 000 ppm (14%; $p < 0.01$, Fisher exact test). The increased mortality in the group at 100 ppm was largely due to the deaths of all 18 pups in one litter. The Meeting considered that this event was not related to treatment.

Male and female F_1 pup body weights were significantly reduced by approximately 7% on postnatal day 21 in the group at 10 000 ppm. A similar response during lactation was observed amongst the F_2 generation pups. On postnatal day 21, the mean body weights in the group at 10 000 ppm were approximately 14% lower for males and 12% lower for female pups and, in the group at 1000 ppm, male pups were approximately 7% lighter than the controls on postnatal day 21. At autopsy of the pups, it was noted that thymus weights were reduced in female F_1 pups of the group at 10 000 ppm (10%), but not in male F_1 pups of any dosed group. In the F_2 pups, thymus weight was not significantly changed in any group of females, while there were significant reductions in all dosed groups of males, by approximately 17%, 12% and 19% in the groups at 100, 1000 and 10 000 ppm, respectively. The absence of a dose–response relationship in males and the absence of any response in females in F2 and the lack of correspondence with observations in F_1 suggested that these thymus responses are not treatment-related. Spleen weights were not affected by treatment in either male or female F_1 pups, while they were reduced in male F_2 pups of the groups at 1000 and 10 000 ppm (17% and 27%, respectively) and in female F_2 pups of the group at 10 000 ppm (18%). These reductions in spleen and thymus weights were not consistent with regard to sex and generation and therefore were not considered to be toxicologically significant.

At the autopsies of the rats in the group at 10 000 ppm, it was found that absolute and relative (to body weight) liver weights were increased in females of the F_0 and the F_1 generations. Absolute and relative liver weights increased by approximately 16% and 21%, respectively in the F_0 generation and by approximately 21% and 21% in the F_1 generation. There were no significant liver-weight changes at 1000 ppm or in male rats of any group. Histology of the liver did, however, reveal changes in both sexes at 1000 and 10 000 ppm (Table 26). Centrilobular hypertrophy occurred in all rats at 10 000 ppm, as well as in significant proportions of rats at 1000 ppm. Hepatocellular degeneration was less common and was not observed at all in any females of the F_1 generation.

Table 26. Incidences of histopathology of the liver in F_0 and F_1 parental rats fed diets containing boscalid

Histopathology	Dietary concentration (ppm)							
	Male rats				Female rats			
	0	100	1000	10 000	0	100	1000	10 000
No. of rats	25	25	25	25	25	25	25	25

F_0 generation parental								
Centrilobular hypertrophy	0	0	9	25	0	0	6	25
Hepatocellular degeneration	0	1	0	3	0	0	0	1
F_1 generation parental								
Centrilobular hypertrophy	1	0	10	25	0	0	8	25
Hepatocellular degeneration	0	0	0	8	0	0	0	0

From Schilling et al. (2001)

A number of other organ-weight changes that were apparently treatment-related were recorded in the parental rats. Absolute spleen weights were reduced in F_0 males and F_1 males and females rats of the groups at 1000 and 10 000 ppm. Absolute kidney weights were reduced in F_1 males and females at 10 000 ppm. While there were several other significant organ-weight changes (brain, ovary), the poor relationship with dose or the lack of consistency suggested that they were not treatment-related. Histological findings in organs of reproduction were incidental and assessed as having been unrelated to treatment.

The NOAEL for toxicity in adult rats was 100 ppm, equal to 10.1 mg/kg bw per day in males and 10.7 mg/kg bw per day in females, on the basis of reduced body-weight gain during lactation, liver-and spleen-weight changes and hepatic histopathology at 1000 ppm, equal to 101.2 mg boscalid/kg bw per day in males and 106.8 mg boscalid/kg bw per day in females. The NOAEL for offspring toxicity was 100 ppm, equal to 10.1 mg/kg bw per day in males and 10.7 mg/kg bw per day in females, on the basis of reduced pup-weight gain and reduced spleen weights (F_2 male pups) at 1000 ppm, equal to 101.2 mg boscalid/kg bw per day in males and 106.8 mg boscalid/kg bw per day in females. There were no effects on reproductive indices at doses up to and including 10 000 ppm, equal to 1034.5 mg boscalid/kg bw per day in males and 1062.0 mg boscalid/kg bw per day in females, the highest dose tested. The Meeting concluded that boscalid is not a reproductive toxicant in rats (Schilling et al., 2001).

(b) Developmental toxicity

Rat

In a study of developmental toxicity, groups of 25 time-mated, female Wistar rats were given boscalid (batch No. N37; purity, 94.4%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day by gavage in aqueous suspension (0.5% Tylose CB 30.000 in distilled water) on days 6–19 of gestation. The day of confirmation of mating (when spermatozoa were detected) was designated day 0 of gestation. The control group of rats received vehicle only. A standard dose volume of 10 ml/kg bw was used. On day 20 of gestation, the females were killed and assessed by gross pathology. Corpora lutea were counted, and the number and distribution of implantation sites were classified. Uteri were examined for live fetuses and intrauterine deaths. The fetuses were weighed, examined for external/visceral abnormalities, sexed, eviscerated and approximately one half were stained for skeletal examination, while the others were examined for soft tissue alterations.

Analytical verification of stability and homogeneity of boscalid in 0.5% Tylose CB 30.000 in distilled water was determined before the start of the study. Verification of test concentrations was performed during the study.

Between 22 and 24 female rats per group became pregnant in the study. Treatment of pregnant Wistar rats with boscalid did not evoke clinical signs of toxicity or cause alterations in food consumption or body-weight gain. There were no treatment-related or biologically relevant differences between the

groups in conception frequencies, mean numbers of corpora lutea, implantation site number or in the values calculated for pre- and postimplantation losses. All differences were within the normal range of deviation expected of rats of this strain and age.

Examination of the fetuses after dissection from the uterus showed normal and expected values unrelated to treatment for the sex distribution, placental weight, fetal weight, external variations and, in general, external malformations. The only external malformations observed were in two fetuses from the group at 100 mg/kg bw per day.

Soft-tissue variations were found in all groups, the more common being unilateral or bilateral dilated renal pelvis and ureter, but their incidence was not treatment-related. Dilated cerebral ventricle was found in one fetus in each of the groups at 100 and 1000 mg/kg bw per day. The mean percentages (± standard deviation) of fetuses per litter with soft tissue variations in the groups at 0, 100, 300 and 1000 mg/kg bw per day were 12.6 ± 14.1%, 17.6 ± 19.4%, 13.0 ± 14.6% and 21.1 ± 18.7%, respectively. The differences were considered to be incidental and all percentages were well within the range for historical controls. These historical means and ranges in a database of 1594 fetuses were: dilated renal pelvis: mean, 13.6%, range, 7.6–21.6%; dilated ureter: mean, 1.4%, range, 0.5–3.6%; total soft-tissue variations: mean, 13.7%, range, 7.6–21.6%. Soft-tissue malformations occurred in single fetuses of each group at 0 and 100 mg/kg bw per day, but none was found in the groups at 300 or 1000 mg/kg bw per day.

Skeletal variations with or without involvement of the associated cartilaginous structures were found in all groups. These involved the skull, ribs, sternum and limbs. The mean percentages of affected fetuses per litter in the groups at 0, 100, 300 and 1000 mg/kg bw per day were 88.8%, 88.3%, 92.3% and 92.9%, respectively. All except one of the skeletal variations appeared to be without any dose–response relationship. The exception was incomplete ossification of the thoracic centra, the incidence of which showed a statistically significant increase (Wilcoxon one-sided test) at 1000 mg/kg bw per day. In spite of this finding, it appeared to be incidental to treatment because no associated evidence for incomplete ossification was found in other parts of the vertebral column. The percentages (± standard deviation) of fetuses per litter with this particular variation at 0, 100, 300 and 1000 mg/kg bw were: 3.0 ± 8.2%, 3.4 ± 8.3%, 1.6 ± 5.1% and 9.2 ± 12.0%. The mean and range for historical controls, based on two experiments carried out in 1997–1998, with updated classification of fetal findings as malformations and variations, were 2.7% and 1.8–3.5%. Skeletal tissue malformations in the form of malpositioned and bipartite ossification of sternebrae (with unchanged cartilage) occurred in individual fetuses of the groups at 0, 100 and 300 mg/kg bw per day, but none was found at 1000 mg/kg bw per day.

Thus under the conditions of this study of prenatal developmental toxicity, the oral administration of boscalid to pregnant Wistar rats from implantation until 1 day before the expected day of parturition (days 6–19 of gestation) evoked no overt maternal toxicity up to the limit dose of 1000 mg/kg bw per day. Furthermore, there were also no substance-induced, dose-related influences on the gestational parameters and no signs of boscalid-induced teratogenicity, up to and including the limit dose of 1000 mg/kg bw per day.

There were no indications for boscalid-induced teratogenicity. The NOAEL for maternal toxicity was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 300 mg/kg bw per day on the basis of reduced ossification of thoracic centra at 1000 mg/kg bw per day (Schilling & Hellwig, 2000b).

Rabbit

In a study of developmental toxicity, groups of 25 artificially inseminated Himalayan rabbits were given boscalid (batch No. N 37; purity, 94.4%) at a dose of 0, 100, 300 or 1000 mg/kg bw in an aqueous suspension (0.5% Tylose CB 30.000 in doubly distilled water) administered by stomach tube

on days 7 to 28 after insemination. A standard dose volume of 10 ml/kg bw was used. The control group received the vehicle only.

Food consumption and body weights were recorded regularly throughout the study period. The state of health of the animals was checked each day.

On day 29 after insemination, all surviving females were killed and assessed by gross pathology (including weight determination of the unopened uterus and the placenta). For each dam, corpora lutea were counted and number and distribution of implantation sites (differentiated as resorptions, live and dead fetuses) were determined. The fetuses were removed from the uterus, sexed, weighed and further investigated for any external, soft tissue and skeletal findings.

The stability of the test substance was proven by reanalysis. The stability and homogeneity of the test substance preparation was analytically verified. The correctness of the concentrations was analytically demonstrated.

Twenty-four to twenty-five females per group became pregnant. Treatment of pregnant Himalayan rabbits with boscalid did not cause mortality. Two deaths occurred as a result of dosing accidents, one in each of the groups at 0 and 300 mg/kg bw per day. Four females were killed after abortions, one in the group at 300 mg/kg bw per day and three at 1000 mg/kg bw per day. In addition, another rabbit in the group at 1000 mg/kg bw per day was killed after premature delivery. While the female from the group at 300 mg/kg bw per day and one of the females from the group at 1000 mg/kg bw per day that aborted showed discoloured faeces and reduced defecation a few days before abortion, the other three rabbits affected in the group at 1000 mg/kg bw per day showed no clinical signs of toxicity. Food consumption was significantly reduced in rabbits at 1000 mg/kg bw per day during several days of treatment, their total food consumption during treatment being approximately 26% less than that of the controls. Slight or occasional instances of reduced food consumption in the other groups were considered to be unrelated to treatment. Reductions in body-weight gain at 1000 mg/kg bw per day were treatment-related. Indeed, there was no body-weight gain in this group over the entire period of dosing, in spite of the pregnancies. At 100 and 300 mg/kg bw per day, body-weight gain was not different from that of the controls. There were no treatment-related differences between the groups with regard to conception frequency, mean numbers of corpora lutea, implantation site number or in the values calculated for pre- and postimplantation losses.

Examination of the fetuses after dissection from the uterus showed normal and expected values unrelated to treatment for sex distribution, placental weight, fetal weight, external variations and, in general, external malformations. The only external malformations observed were in one fetus from the group at 100 mg/kg bw per day and two fetuses from the group at 100 mg/kg bw per day. None were found at 0 or 1000 mg/kg bw per day.

Soft-tissue variations were found in all groups, the more common being malpositioned carotid branch, which is a very common finding in Himalayan rabbits. Their incidence showed no relationship to treatment. The mean numbers (± standard deviation) of affected fetuses per litter in the groups at 0, 100, 300 and 1000 mg/kg bw per day were: 5.3 ± 9.4, 7.3 ± 11.3, 6.1 ± 16.2 and 7.5 ± 10.9, respectively. Soft-tissue malformations occurred in some fetuses in each of the groups at 0, 100 and 300 mg/kg bw, but none were found at 1000 mg/kg bw per day.

Skeletal variations with or without involvement of the associated cartilaginous structures were found in all groups. These involved the skull, ribs, sternum and limbs. All except one of the skeletal variations appeared to be without any dose–response relationship. The exception was incomplete ossification of the thoracic centra, the incidence of which was statistically significantly increased (Wilcoxon one-sided test) in the group at 1000 mg/kg bw per day. In spite of this finding, it was considered to be incidental to treatment because no associated evidence for incomplete ossification was found in other parts of the vertebral column. The percentages (± standard deviation) of fetuses per litter with this particular variation in the groups at 0, 100, 300 and 1000 mg/kg bw per day were:

0.7 ± 3.5%, 1.8 ± 4.2%, 2.9 ± 9.0% and 8.3 ± 17.2%. The mean and range for historical controls, based on two experiments carried out in 1997–1998, with updated classification of fetal findings as malformations and variations, were 0.1% and 0.0–0.5%, respectively.

Skeletal tissue malformations (the most common being defect of the cardiac muscular ventricular septum) occurred in individual fetuses in the groups at 0, 100 and 300 mg/kg bw per day, but none was found at 1000 mg/kg bw per day.

There were no indications that boscalid induced teratogenicity. The NOAEL for maternal toxicity was 300 mg/kg bw per day on the basis of reduced feed consumption and body-weight gain at 1000 mg/kg bw per day. The NOAEL for developmental toxicity was 300 mg/kg bw per day on the basis of reduced ossification of thoracic centra at 1000 mg/kg bw per day.

The Meeting concluded that boscalid is unlikely to be teratogenic to man (Schilling & Hellwig, 2000a).

2.6 Special studies

(a) Neurotoxicity

(i) Neurotoxic potential

In a single-dose study of neurotoxicity, groups of 10 male and 10 female Wistar rats were given boscalid (batch No. N 46; purity, 96.3%) as a single oral dose at 0, 500, 1000 or 2000 mg/kg bw by gavage. The vehicle was a 0.5% aqueous solution of Tylose CB 30.000, and the dose administration volume was 20 ml/kg bw.

The rats were observed for up to 2 weeks after dosing. Their general state of health was examined and recorded daily. Body weight was determined 7 days before dosing, on day 0 (administration of test substance) and on days 7 and 14. Functional observational batteries (FOB) and motor activity measurements were carried out on all rats on the same days. The measurements for day 0 were made within a few hours after dosing. Five rats per sex and dose were killed, fixed by in-situ perfusion and subjected to neuropathological examinations. The remaining animals were killed with CO_2 under anaesthesia without any further examinations. The stability, homogeneity and correctness of the concentrations of the boscalid preparation were verified analytically.

There were no deaths during the study and the only clinical sign of toxicity that was treatment-related was piloerection in two female rats at 2000 mg/kg bw during the FOB on day 0. There were no other test-substance related effects at any dose. In particular, no signs of neurotoxicity were observed. The NOAEL for neurotoxicity after a single oral dose of boscalid was 2000 mg/kg bw in male and female rats (Mellert et al., 2000d).

In a multiple-dose study of neurotoxicity, groups of 10 male and 10 female Wistar rats were given diets containing boscalid (batch No. N 46; purity, 96.3%) at a concentration of 0, 150, 1500 or 15 000 ppm, equal to 0, 10.5, 103 and 1050 mg/kg bw per day in males and 0, 12.7, 124.5 and 1273 mg/kg bw per day in females, for 3 months.

Food and water consumption were determined once per week. Body weight was determined once per week and on the days when FOB were performed. A check of the general state of health was made at least daily. Furthermore, the rats were thoroughly examined and palpated once per week. FOB and measurements of motor activity were carried out in all animals 7 days before the start of the administration and on days 22, 50 and 85. Five rats per sex and dose were killed, then fixed by in-situ perfusion and subjected to neuropathological examinations. The remaining animals were killed with CO_2 under anaesthesia without any further examinations. The

stability, homogeneity and correctness of the concentrations of boscalid in the diet were verified analytically.

There were no adverse effects related to treatment at any dose. In particular, there were no signs of neurotoxicity at any dose. The NOAEL for neurotoxicity was 15 000 ppm, equal to 1050 mg/kg bw in males and 1272 mg/kg bw in females, the highest dose tested (Mellert et al., 2001c).

(ii) Delayed neurotoxicity in hens

As there were no neurotoxic effects observed in any of the experiments with boscalid, studies on delayed neurotoxicity in hens were not performed.

(iii) Developmental neurotoxicity in rats

Boscalid (batch No. N 46; purity, 96.3%) was tested for its effect on the embryonic, fetal and postnatal development of the nervous system in Wistar rats in this developmental study of neurotoxicity. Groups of 35 mated female Wistar rats were given boscalid at a concentration of 0, 100, 1000 or 10 000 ppm, equal to 0, 14, 147 and 1442 mg/kg bw per day, continuously as a homogeneous addition to the feed from day 6 postcoitum to postnatal day 21. The dams were allowed to litter and rear their offspring until day 4 (standardization of litters) or 21 after parturition. After the offspring had been weaned following lactation until day 21, the dams were killed and not examined further.

The state of health of the dams and offspring was evaluated each day. Food consumption and body weights of the dams were determined on days 0, 6, 13 and 20 of gestation and on days 1, 7, 14 and (body weight only) 21 of lactation. A detailed clinical examination outside the cage (open-field observations) was made on selected dams on days 7 and 14 of gestation and on days 7 and 14 of lactation.

The offspring were sexed and examined for macroscopically evident changes on the day of birth. They were weighed on the day after birth and on postnatal days 4, 11, 17 and 21 and after weaning, once per week at weekly intervals. Their viability was recorded. Sexual maturation (day of preputial separation/vaginal opening) of all selected offspring and their body weights were determined and recorded. A detailed clinical examination outside the cage (open field observations), measurements of motor activity and tests for auditory startle and learning and memory (water-maze test) were performed on selected offspring. All offspring not required for any of these examinations were killed and discarded without further examinations. Neuropathological examinations and determinations were carried out on selected offspring on days 11 and 60 after birth. The stability, homogeneity and concentrations of boscalid in the diet were confirmed analytically.

There were no treatment-related effects on parental females at any dose. Offspring of the group at 10 000 ppm showed slightly reduced mean body weights in males and females throughout lactation and reduced mean body-weight gain on postnatal days 1–4 and 17–21. The latter was about 5% below body-weight gain in the control group, if calculated for postnatal days 4–21. Mean body weights and body-weight gains were also reduced in the group at 1000 ppm during the early postnatal days, but there were no body-weight deficits at 100 ppm. Absolute weights of the brain and the length of the brain (male rats) were reduced only in those rats killed on postnatal day 11 at 10 000 ppm. These brain-weight and length effects were considered to be consequences of the retarded body-weight development. No similar changes were found in the groups at 100 or 1000 ppm. There were no signs of developmental neurotoxicity at any dose.

The NOAEL for maternal toxicity was 10 000 ppm, equal to 1442 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 100 ppm, equal to 14 mg/kg bw per day, on the basis of reduced body weights of pups during lactation at 1000 ppm, equal to 147 mg/kg bw per day. The reduction in pup body weight at the two higher doses was consistent with similar effects seen in the two-generation study in rats in which the same doses induced hepatotoxicity.

In this study of developmental neurotoxicity, boscalid had no adverse effects on the embryonic, fetal and postnatal development of the nervous system in Wistar rats at doses of up to 10 000 ppm, equal to 1442 mg/kg bw per day, the highest dose tested (Kaufmann et al., 2001). The Meeting concluded that boscalid is not neurotoxic in adult or developing rats.

The Meeting concluded that boscalid is unlikely to cause neurotoxicity in human beings.

(b) Liver and thyroid effects

The potential of boscalid (batch No. N 26; purity, 95.3%) to induce hepatic metabolizing enzymes was investigated in rats. Groups of five male and five female Wistar rats were given boscalid at a dietary concentration of 0 or 15 000 ppm for 2 weeks and a number of hepatic parameters were measured. Additional groups of three male and three female rats were also treated for 2 weeks with the same diets and subjected to perfusion fixation for subsequent electron microscopy of the liver. The stability of boscalid in the diet, the homogeneity of the dietary mixture and the correctness of the concentration were demonstrated.

Liver weights were increased by 32% in males and 23% in females at 15 000 ppm and light and electron microscopy demonstrated a centrilobular (zone 3) proliferation of smooth endoplasmic reticulum. In hepatocytes showing gross proliferation of smooth endoplasmic reticulum, glycogen storage was depleted. The only biochemical parameter showing a significant change in both sexes was the content of cytochrome P450, which was increased in male rats by 124% and in female rats by 74%. Lipid peroxidation was slightly increased in males, but not in females.

Parameters in rat liver that showed no significant differences between the groups at 0 and 15 000 ppm for either sex were: cyanide-insensitive palmitoyl-CoA oxidation, ethoxyresorufin-O-deethylase, pentoxyresorufin-O-dependentylase, and glutathione. In the absence of a change in glutathione concentration, the small change in lipid peroxidation activity in male rats only is of doubtful toxicological significance (Mellert et al., 1999). The Meeting concluded that boscalid induces proliferation of smooth endoplasmic reticulum and rat liver cytochrome P450 enzymes, although no specific isoenzymes had been identified.

Organ-specific effects of boscalid were extended to the thyroid, in view of the thyroid toxicity and possible neoplasia observed in a long-term experiment described earlier in the present monograph. It is known that possible modes of action on thyroid can involve the liver, so investigations in this study were extended to this organ, to supplement the measurements in the experiment previously described. Groups of five male and five female Wistar rats were fed diets containing boscalid (batch No. N 46; purity, 96.3%) at a concentration of 0 or 15 000 ppm, equal to 0 and 957 mg/kg bw per day for males and 0 and 1197 mg/kg bw per day for females for 4 weeks. Food consumption and body weights were determined once per week and the rats were examined for signs of toxicity or mortality at least once each day. Thyroid hormone analyses (T3, T4, TSH) were carried out on study days −3, 2, 4, 7, 14, 21 and 28. So-called phase II liver enzyme activities (*p*-nitrophenol-glucuronyltransferase, 4-methylumbeliferone-glucuronyltransferase and 4-hydroxybiphenyl-glucuronyltransferase) were measured in liver at the end of the study. The stability of the test substance over the study period was demonstrated. The stability of boscalid in the diet, the homogeneity of the dietary mixture and the correctness of the concentration were demonstrated.

There were no clinical signs of toxicity and no effects on body weight or food consumption at 15 000 ppm. Total T3 concentration was lower by approximately 15–30% of control values in male rats at 15 000 ppm throughout the study. With the exception of day 4, all these changes were seen as a trend toward reduced values. In females, reductions in the concentration of T3 of 1–25% from day 4 onwards were not statistically significant.

Total concentrations of T4 were lower by approximately 13–27% of control values in male rats at 15 000 ppm from day 4 onwards. On day 2, this finding was also seen as a trend toward reduced values. In the treated females, serum concentrations of T4 were slightly reduced by approximately 3–13% of control values from day 7 onwards, without being statistically significantly different to the control values. With the exception of day 4, all these changes were seen as a trend toward reduced values.

Concentrations of TSH were statistically significantly increased (approximately 170–280% of control values) in males at 15 000 ppm from day 14 onwards. From day 2 until day 7, this finding occurred as a trend toward increased values. With the exception of day 7, statistically significantly increased concentrations of TSH of approximately 180–280% of control values were found in the sera of the treated females throughout the study.

Liver weights were statistically significantly increased by 25% and 22% in treated males and females, respectively. *p*-Nitrophenol-glucuronyltransferase activities in the liver were statistically significantly increased in treated males and females by almost 2-fold and 1.25-fold, respectively. 4-Methylumbeliferone-glucuronyltransferase activities were statistically significantly increased in treated males and females by 2-fold and 2.4-fold, respectively. 4-Hydroxybiphenyl-glucuronyltransferase activities were statistically significantly increased in treated males and females by almost threefold in both sexes. Thus, the activities of all of the hepatic glucuronyltransferases examined were increased in male and female rats receiving boscalid at 15 000 ppm for 4 weeks. It is reasonable to assume that increased conjugation of T4 was responsible for the increase in circulating concentrations of TSH (Mellert et al., 2001e).

(c) Immunotoxicity

In a test for immunotoxicity, groups of 16 male Wistar rats were given diets containing boscalid (batch No. N37; purity, 94.4%) at a concentration of 0, 100, 1000 or 10 000 ppm, equal to 0, 7.5, 73.1 and 736.2 mg/kg bw per day for 4 weeks. These concentrations were chosen in accordance with those of the two-generation study in rats described above. The rats were about 6 weeks of age when first exposed to boscalid. As a positive control, groups of 16 male Wistar rats were given cyclophosphamide at a dose of 0 (0.5% methyl cellulose solution as vehicle control) and 3 mg/kg bw per day by gavage. Rats were observed daily for clinical signs of toxicity. Food consumption and body weight were recorded. Eight rats of each group were selected for flow cytometry analysis of lymphocytes for anti-sheep-erythrocyte immunoglobulin M (IgM) measurement at the end of the dosing period.

One rat at 10 000 ppm was killed in extremis; otherwise, there were no clinical signs of toxicity or mortalities. In the groups treated with boscalid or cyclophosphamide, food consumption and body weights were comparable to those of the negative control group throughout the treatment period. There were no significant changes in the weights of either the spleen or thymus, and cell numbers in the thymus and spleen were unaltered by treatment. Flow cytometric analysis of lymphocyte subsets from thymus and spleen did not reveal significant differences. A significant increase in the Pan T-cell subset of rats in the group at 100 ppm was noted as an incidental finding with no dose–response relationship, since no similar response was observed at 1000 or 10 000 ppm. There were no statistically significant differences in the serum anti-sheep-erythrocyte IgM antibody titres between the treatment group and control group. In contrast, treatment with cyclophosphamide caused significant decreases in the weight and cell numbers of spleen and thymus, decreases in all the splenic lymphocyte subsets and anti-sheep-erythrocyte IgM antibody titre. The results of this study showed that boscalid does not induce immunotoxicity in rats exposed at dietary concentrations of up to 10 000 ppm, equal to 736.2 mg/kg bw per day, for 4 weeks (Kosaka, 2003).

3. Observations in humans

In the short time since industrial production of boscalid has started, no occupational incidence reports suggesting substance-related adverse effects in personnel engaged in the production of active substance or formulation of preparations have been brought to the attention of the medical department of BASF Aktiengesellschaft.

Comments

Biochemical aspects

In rats given [^{14}C]boscalid as a single oral dose at 50 or 500 mg/kg bw, the radiolabel was rapidly but incompletely absorbed from the gastrointestinal tract, widely distributed, and rapidly eliminated from the body. The excretion balance of boscalid demonstrates that at doses of 50 and 500 mg/kg bw approximately 16% and 3%, respectively, of the administered is excreted via the urine. Excretion via the faeces accounted for 80% (lowest dose) to 95% (highest dose) of the dose; however, 40% of the lowest dose and 12% of the highest dose was eliminated from the body via the bile. Bioavailability decreased with increasing dose, and was estimated to be about 50% at the lowest dose, but only about 15% at the highest dose. The plasma concentration–time curve showed two maxima. The initial half-life was approximately 7–8 h and the terminal half-life was approximately 20–40 h. Tissue distribution determination showed that the largest amounts of radioactivity were in the gastrointestinal tract, liver and adipose tissue. There is no evidence for a cumulative potential of boscalid.

The dermal absorption of boscalid in rats in vivo is approximately 8% or less, depending on the duration of exposure and concentration applied. The initial rate of penetration through rat epidermal membranes was at least 7.7-fold greater than through human epidermal membranes. When the amount of radioactivity remaining associated with the skin was also taken into account, it was concluded that there is no notable difference between rats and humans in the bioavailability of boscalid applied to the skin.

After oral administration to male and female rats, the systemically available portion of boscalid was rapidly and extensively metabolized to a large number of biotransformation products. The hydroxylation of the diphenyl moiety was the quantitatively most important pathway. The second important pathway was the substitution of the chlorine of 2-chloropyridine by conjugation with glutathione. Partial cleavage of the glutathione moiety afforded the cysteine conjugate and finally the sulfhydryl compound that was subsequently methylated or oxidized. In addition, the introduction of glutathione and a second hydroxyl group into the diphenyl moiety was observed. Combinations of these reactions and the conjugation of the hydroxyl groups with glucuronic acid or sulfate, and the conjugation of the sulfhydryl group with glucuronic acid led to the large number of metabolites that have been identified. Cleavage of the amide bond appeared to be negligible because 2-chloronicotinic acid was detected only in trace amounts. No major differences were observed with regard to label, sex or dose.

Toxicological data

In male and female rats, the toxicity of boscalid was low, with values for the oral LD_{50} of > 5000 mg/kg bw, dermal LD_{50} of > 2000 mg/kg bw and inhalation LC_{50} of > 6.7 mg/l. Furthermore, boscalid is not irritating to the skin or the eyes of rabbits. In a maximization test for sensitizing potential in guinea-pigs, about one fifth of the animals developed a reaction upon challenge.

Overall, in short-term studies with boscalid, the signs of toxicity observed in mice, rats and dogs were similar, with reduced body-weight gain, changes in clinical chemistry, and liver enlargement being common features. The results of the short-term studies suggest that the increased liver weights were indicative of an adaptive response to exposure. Histopathology confirmed the liver as a target organ with observation of hypertrophy of hepatocytes, although this too is indicative of an adaptive response. Hypertrophy was accompanied by proliferation of smooth endoplasmic reticulum and the induction of cytochrome P450 protein (although no increased activities of specific CYP isoenzymes were identified) and several microsomal enzymes involved in conjugation reactions. The rat was the only species investigated for these particular effects. There were also increases in blood gamma-glutamyltransferase activity at higher doses. Furthermore, in mice and rats given diets containing high concentrations of boscalid there was increased lipid accumulation in the liver. Absolute thyroid weights were increased in male and female rats of some groups at higher doses, but not in dogs in which there were, however, some increases in relative weight of the thyroid. In male rats there was an increased incidence of thyroid follicular cell hyperplasia at doses of 2000 ppm, equal to 139 mg/kg bw, and higher, but this did not occur in female rats or in dogs of either sex. There is evidence that this effect is due to excessive perturbation of the pituitary–hypothalamus–thyroid-axis homeostatic mechanism, consisting of increases in the activity of hepatic glucuronyltransferase enzymes, some of which are involved in the metabolism of thyroid hormones, and in decreases in the concentrations of circulating thyroid hormones.

The NOAELs for the short-term dietary studies were: 90-day study in rats, 500 ppm (equal to 34 mg/kg bw per day); 90-day dietary study in mice, 150 ppm (equal to 29 mg/kg bw per day); 90-day study in dogs, 250 ppm (equal to 7.6 mg/kg bw per day); 12-month study in dogs, 800 ppm (equal to 22 mg/kg bw per day). In a 4-week study of dermal toxicity with boscalid in rats, no substance-related systemic findings were detected at 1000 mg/kg bw per day, the highest dose tested.

Long-term feeding studies with boscalid in rats and mice confirmed that the primary target organ was the liver. There was no evidence for a carcinogenic potential in mice, and a higher incidence of thyroid follicular cell tumours in rats was due to benign neoplasms, the incidence of these tumours being not statistically significantly different from that in the control group. Since no clear increase in the incidence of thyroid follicular cell tumours was observed, it was not considered necessary to investigate modes of action in a formal or detailed manner. In addition, the $BMDL_{10}$ for thyroid adenomas in male rats, according to a number of statistical models, was 76 mg/kg bw per day. This demonstration, that very high doses are required to produce proliferative responses, even in rats, reinforces the conclusion drawn from the mechanistic studies, i.e. that this is exclusively a phenomenon that occurs at high doses and is not relevant for human exposure. The NOAEL identified in long-term studies in rats was 100 ppm, equal to 4.4 mg/kg bw per day, on the basis of increased gamma-glutamyltransferase activity and hepatic centrilobular hypertrophy and eosinophilic foci in males and increased concentration of cholesterol and reduced prothrombin times in females at 500 ppm, which may be considered as an LOAEL. In long-term studies in mice, the NOAEL was 80 ppm (equal to 13 mg/kg bw per day) on the basis of decreased body-weight gain in males.

Boscalid was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence for genotoxicity was observed in any test. The Meeting concluded that boscalid is unlikely to be genotoxic.

In the absence of genotoxicity and any evidence of relevant carcinogenicity in rodents, the Meeting concluded that boscalid is unlikely to pose a carcinogenic risk to humans.

The reproductive toxicity of boscalid was investigated in a two-generation study of reproduction in rats and in studies of developmental toxicity in rats and rabbits.

Reproductive function was not affected in rats in the two-generation study with boscalid and the NOAEL for reproductive function was 10 000 ppm, equal to 1034 mg/kg bw per day, the highest dose tested. The NOAEL for systemic toxicity in the parental animals in the two-generation study

was 100 ppm, equal to 10 mg/kg bw per day on the basis of reduced body-weight gain, liver-weight increases, spleen-weight decreases and increased incidences of hepatic centrilobular hypertrophy at 1000 ppm, equal to 101 mg/kg bw per day. In pups, decreased body-weight gain and reduced spleen weights at 1000 ppm were the effects that defined the NOAEL for offspring toxicity at 100 ppm, equal to 10 mg/kg bw per day. In the two-generation study of toxicity in rats, there were reductions in spleen and thymus weight in the pups, but these were not consistent with regard to sex and generation and therefore not considered to be toxicologically significant.

In a study of developmental toxicity, rats were given boscalid at doses of up to 1000 mg/kg bw per day. No maternal toxicity was observed. There was an increased incidence of delayed ossifications (of thoracic centra), which was statistically significantly increased at 1000 mg/kg bw and exceeded the range for historical controls. The NOAEL for maternal toxicity was 1000 mg/kg bw per day, the highest dose tested, and the NOAEL for developmental toxicity was 300 mg/kg bw on the basis of reduced ossification of thoracic centra.

In a study of developmental toxicity in rabbits given boscalid at doses of up to 1000 mg/kg bw per day, significant maternal toxicity, consisting of reduced feed consumption and body-weight gain, was observed at the highest dose. There was an increased incidence of delayed ossifications (of thoracic centra) at 1000 mg/kg bw per day. Both the observed litter incidence of delayed ossifications and the number of affected fetuses per litter were statistically significantly increased above control levels and clearly exceeded the range for historical controls. The increase in fetal incidence was dose-related and exceeded the range for historical controls in all groups, including the controls. The NOAEL for maternal toxicity was 300 mg/kg bw per day and the NOAEL for developmental toxicity was 300 mg/kg bw per day on the basis of reduced ossification of thoracic centra.

No signs of neurotoxicity were noted in any studies, including specific studies for neurotoxicity.

There was no evidence of immunotoxicity in a study for immunotoxicity in adult rats exposed to boscalid at dietary concentrations of up to 10 000 ppm, equal to 736.2 mg/kg bw per day for 4 weeks.

The Meeting concluded that the existing data were adequate to characterize the potential hazard to fetuses, infants and children.

Toxicological evaluation

An ADI of 0–0.04 mg/kg bw was established for boscalid based on the NOAEL of 4.4 mg/kg bw per day, identified on the basis of increased gamma-glutamyltransferase activity and increased incidences of hepatic eosinophilic foci in male rats in a 24-month long-term dietary study of toxicity and carcinogenicity and a safety factor of 100.

The Meeting concluded that it was not necessary to establish an ARfD for boscalid in view of the well-demonstrated lack of toxicity in studies of acute toxicity, the absence of relevant developmental toxicity that could have occurred as a consequence of a single exposure, the absence of any indication of neurotoxicity and the absence of any other adverse effects that would be likely to be induced after a single or a small number of exposures in repeat-dose studies.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month study of toxicity and carcinogenicity	Toxicity	80 ppm, equal to 13 mg/kg bw per day	400 ppm, equal to 65 mg/kg bw per day
		Carcinogenicity	8000 ppm[a], equal to 1345 mg/kg bw per day	—

Rat	24-month studies of toxicity and carcinogenicity	Toxicity	100 ppm, equal to 4.4 mg/kg bw per day	500ppm, equal to 23 mg/kg bw per day
		Carcinogenicity	2500 ppm[a], equal to 116 mg/kg bw per day	—
	Two-generation study of reproductive toxicity[b]	Reproductive toxicity	10000 ppm[a] equal to 1034 mg/kg bw per day	—
		Parental toxicity	100 ppm, equal to 10 mg/kg bw per day	1000 ppm, equal to 101 mg/kg bw per day
		Offspring toxicity	100 ppm, equal to 10 mg/kg bw per day	1000 ppm, equivalent to 101 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	1000 mg/kg bw per day[a]	—
		Embryo and fetal toxicity	300 mg/kg bw[a] per day	1000 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	300 mg/kg bw per day	1000 mg/kg bw per day
		Embryo and fetal toxicity	300 mg/kg bw per day	1000 mg/kg bw per day
Dog	1-year study of toxicity	Toxicity	800 ppm, equal to 22 mg/kg bw per day	2000 ppm, equal to 57 mg/kg bw per day

[a] Highest dose tested

[b] Measurements of intake of the compound are the mean of the premating phases for F_0 and F_1 females

[c] Gavage administration

Estimate of acceptable daily intake for humans

0–0.04 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 boscalid

Absorption, distribution, excretion and metabolism	
Rate and extent of oral absorption	High, inverse dose-dependent bioavailability of 50–15%; two plasma T_{max} values, 1 h and 8h (rat)
Dermal absorption	Approximately 8% (rat)
Distribution	Distributed throughout the body; higher concentrations in liver and gastrointestinal tract
Potential for accumulation	No evidence
Rate and extent of excretion	High (but determined by the low rate of absorption); essentially 100% excretion in bile and urine within 168 h,
Metabolism in animals	Extensive, about 40 metabolites, little parent compound remaining
Toxicologically significant compounds (animals, plants and environment)	Parent

Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 6.7 mg/l air (4 h)
Rabbit, LD_{50}, dermal	> 2000 mg/kg bw
Rabbit, skin irritation	Not irritating
Rabbit, eye irritation	Not irritating
Guinea-pig, skin sensitization	Not sensitizing
Short-term studies of toxicity	
Target/critical effect	Liver, thyroid; body weight
Lowest relevant oral NOAEL	22 mg/kg bw per day (12-month study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (4-week study in rats)
Lowest relevant inhalation NOAEC	No data
Genotoxicity	Not genotoxic in vivo or in vitro
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver, thyroid; body weight
Lowest relevant NOAEL	4.4 mg/kg bw per day (24-month study in rats)
Carcinogenicity	Boscalid induced a low incidence of benign thyroid tumours in rats
Reproductive toxicity	
Reproductive target/critical effect	None
Lowest relevant reproductive NOAEL	1034 mg/kg bw[a,b] per day
Developmental target/critical effect	Not teratogenic; reduced fetal body weight, delayed ossifications
Lowest relevant developmental NOAEL	300 mg/kg bw[a] per day (rat, rabbit)
Neurotoxicity/delayed neurotoxicity	
	No signs of neurotoxicity
Other toxicological studies	
	Liver xenobiotic metabolizing enzyme induction.
Medical data	
	No reports of toxicity in workers exposed during manufacture or use

Summary

	Value	**Study**	**Safety factor**
ADI	0–0.04 mg/kg bw	Rat, 2-year study of toxicity and carcinogenicity	100
ARfD	Unnecessary	—	—

[a] Highest dose tested

[b] Measurements of intake of the compound are the mean of the pre-mating phases for P and F_1 females

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CYFLUTHRIN AND BETA-CYFLUTHRIN

First draft prepared by
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Explanation

Cyfluthrin, the International Organization of Standardization (ISO) approved common name for 3-(2,2-dichloro-vinyl)-2,2-dimethyl-cyclopropane-carboxylic acid cyano-(4-fluoro-3-phenoxy-phenyl)-methyl ester(*RS*), is a synthetic cyano-containing pyrethroid insecticide. It was evaluated by the JMPR in 1987, when an acceptable daily intake (ADI) of 0–0.02 mg/kg bw was established based on a no-observed-adverse-effect level (NOAEL) of 50 ppm, equal to 2 mg/kg bw per day, which was identified on the basis of reduced body-weight gains in a 2-year study in rats and using a safety factor of 100. In 1997, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) also established an ADI of 0–0.02 mg/kg bw.

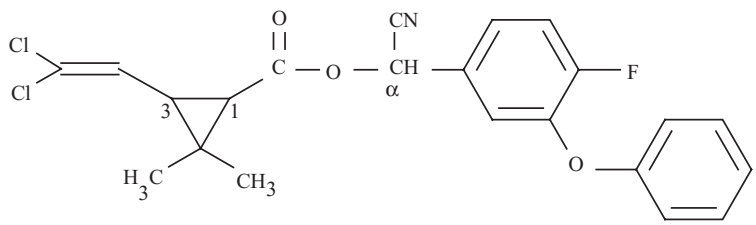
Technical-grade cyfluthrin consists of a mixture of four diastereomeric pairs of enantiomers (giving rise to eight optical isomers), consisting of two *cis* and two *trans* isomeric pairs. Beta-cyfluthrin consists of two diastereoisomeric pairs, which are the biologically active isomers of cyfluthrin. They are contained in cyfluthrin at a concentration of about 40%.

Cyfluthrin was re-evaluated by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR). Beta-cyfluthrin has not been evaluated previously. The Meeting reviewed new data on cyfluthrin and beta-cyfluthrin that had not been considered previously, and relevant data from the previous evaluation.

For cyfluthrin, the specifications were established by the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS) and published as *WHO specifications and evaluations for public health pesticides*: *cyfluthrin* (2004).[1]

Not all pivotal studies with cyfluthrin were certified as being compliant with good laboratory practice (GLP). These studies were carried out before the Organisation for Economic Co-operation and Development (OECD) guidelines on GLP were promulgated. However, the quality of these studies was considered to be acceptable.

Figure 1. Chemical structure of beta-cyfluthrin



[1] Available from http://www.who.int/whopes/quality/en/Cyfluthrin_spec_eval_WHO_Nov_2004.pdf

Table 1. Diastereoisomer pairs of cyfluthrin and beta-cyfluthrin

Pair No.	Cyfluthrin (FCR 1272)	Beta-cyfluthrin
I	1R - 3R - αR	—
	1S - 3S - αS	
II	1R - 3R - αS	—
	1S - 3S - αR	
III	1R - 3S - αR	Beta-cyfluthrin (FCR 4545)
	1S - 3R - αS	
IV	1R - 3S - αS	—
	1S - 3R - αR	

Evaluation for acceptable daily intake

Cyfluthrin

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

Groups of fasted male Wistar rats received cyfluthrin (purity unknown; FCR 1272, cyfluthrin isomer ratio: I, 26.6%; II, 19.1%; III, 33.7%; IV, 20.6%) as a single oral dose at 10 mg/kg bw, dissolved in either polyethylene glycol (PEG 400) or a Cremophor EL : water emulsion. Two rats per group were killed at 0.5, 1, 2, 4, 6, 16 and 24 h after treatment. The concentrations of the respective enantiomers of cyfluthrin were assessed in blood and the stomach.

Cyfluthrin in Cremophor EL : water emulsion was rapidly absorbed. The four enantiomers were already detectable in the blood after 30 min and peak concentrations in blood were reached after 1 h. The absorption of cyfluthrin in PEG 400 was slower; detectable concentrations in blood were found after 4 h and peak blood concentration was reached after 6 h. Peak concentrations with PEG 400 were five times lower than with the Cremophor EL : water emulsion. In the group receiving Cremophor EL : water emulsion, only small amounts of cyfluthrin were detected in the stomach 4 h after administration, while in the group receiving PEG 400 considerably higher amounts were detected in the stomach at this time. In both groups the *cis : trans* isomer ratio in the blood shifted in favour of the *cis*-enantiomer, indicating that the *trans*-enantiomer is broken down more rapidly than the *cis*-enantiomer (Eben et al., 1982).

Groups of five male and five female Mura:SPRA (SPF 68 Han) rats received ^{14}C-labelled cyfluthrin (radiochemical purity, 98%, labelled in the fluorobenzene moiety) either as single oral doses at 0.5 and 10 mg/kg bw, or as single intravenous doses at 0.5 mg/kg bw. A group of five male that were equipped with bile-duct cannula received cyfluthrin at a dose of 0.5 mg/kg bw intraduodenally. Additional groups of five male and five females received unlabelled cyfluthrin as 14 daily oral doses at 0.5 mg/kg bw, followed by ^{14}C-labelled cyfluthrin at the same dose. Samples of urine, faeces, organs, tissues and blood were collected at several time-points after dosing.

After oral administration of 0.5 or 10 mg/kg bw, about 90% was absorbed (in females in the group at 10 mg/kg bw, only 80%) The t½ for absorption was about 30 min. About 98% of the radioactivity was excreted within 48 h, with a rapid (t½ of 2–3 h) and slow phase (t½ of 9–12 h). The urine : faeces ratio of excretion was higher in males (3 : 1) than in females (3 : 2). Less than 0.001% was excreted through expired air. No marked differences in kinetics between the groups at the lower and higher doses were observed.

After intravenous administration, biphasic elimination was found, with a t½ of 2 h for the rapid phase and a t½ of 20 h for the slow phase. Forty-eight hours after intravenous administration; 93–95% of the dose was excreted, again with a higher urine : faeces ratio in males than in females. In male rats with bile-duct cannulations, after an intraduodenal dose at 0.5 mg/kg bw, about 33% of radioactivity was excreted in the bile within 2 days, about half of which was excreted within 2 h.

The apparent volume of distribution was about 17%, which is consistent with distribution primarily in extracellular fluid. The highest concentrations were found in fat, ovaries, adrenal, liver and spleen. The lowest concentrations were found in the brain.

Daily dosing for 14 days with unlabelled cyfluthrin followed by a single dose of [^{14}C]-cyfluthrin on day 15 yielded results similar to those of the single-dose experiments (Klein et al., 1983)

Groups of male Mura:SPRA (SPF 68 Han) rats received oral doses of [^{14}C]-cyfluthrin (radiochemical purity, 98%, labelled in the fluorobenzene moiety) at 0.5 or 10 mg/kg bw, or 0.5 mg/kg bw via the intravenous or intraduodenal route. Rats treated intraduodenally were fitted with bile-duct cannula. In addition a group of female rats received a single oral dose of cyfluthrin at 0.5 mg/kg bw.

After oral administration, about 90% was absorbed, with a half-life of about 0.5 h. Maximum concentrations in blood were reached after 1.5 h. Thereafter, in the groups at the lower or higher dose, similar declines in plasma concentrations were observed. In female rats, plasma concentrations were 1.5 to 3 times higher than in male rats at the lower dose. Two and 10 days after the oral administration, respectively 1–2% and 0.2% remained in the body. At day 10, the highest concentrations were found in the fat. Low concentrations were detected in the spleen, testes, erythrocytes and plasma. Excretion through expiration was less than 0.001%. In rats with bile-duct cannula, after an intraduodenal dose of 0.5 mg/kg bw, about one third of the radioactivity was excreted in the bile within 2 days, 50% and 90% of which was excreted within 2 and 6 h respectively.

After intravenous administration, 94% of radioactivity was excreted within 48 h (70% in the urine, 24% in the faeces). The volume of distribution was 18% (Weber & Suwelack, 1983)

1.2 Biotransformation

The studies on the metabolism of cyfluthrin in animals using radioactively labelled material were restricted to the ^{14}C-phenyl- or ^{14}C-fluorophenyl-labelled parent compound. The metabolite 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCVA) that would be released after the cleavage of the ester bond had been extensively investigated in mice, rats, cows and hens as a part of studies on other chemically similar synthetic pyrethroids. This acid portion has been reported to undergo hydroxylation at the methyl groups. DCVA and its hydroxylated derivative were found to form various conjugates in mammalian and avian systems (Casida et al., 1979; Miyamoto et al., 1981). The results can be extrapolated to cyfluthrin.

(i) In vivo

Rats

Four groups of five male and five female Sprague Dawley (SPF 68 Han) rats received [^{14}C]-cyfluthrin (radiochemical purity, 98%) either as an oral dose at 0.5 or 10 mg/kg bw or as an intravenous dose at 0.5 mg/kg bw. Another group received unlabelled cyfluthrin orally once per day for 14 consecutive days, followed by a single oral dose of [^{14}C]-cyfluthrin at 0.5 mg/kg bw. For identification of metabolites, samples of urine and faeces were collected at 8 h (urine only), 24 h and 48 h after dosing.

The initial step in the biotransformation of cyfluthrin was ester hydrolysis, giving a 3-phenoxy-4-fluorobenzyl alcohol intermediate and the permethric acid fraction. The metabolism of permethric acid has been well established in studies with chemically similar pyrethroids in rats. After ester hydrolysis, the 3-phenoxy-4-fluorobenzyl alcohol moiety was oxidized to the free metabolite 3-phenoxy-4-fluorobenzoic acid. This metabolite can then either be conjugated with glycine to form 3-phenoxy-4-fluorohippuric acid or hydroxylated to give 4′-hydroxy-3-phenoxy-4-fluorobenzoic acid (conjugates of which account for 41–50% of the total urinary radiolabel recovered from rats given one or multiple doses of cyfluthrin at 0.5 mg/kg bw). Females tended to excrete more of this metabolite as the free form in the faeces than did males. Males and females at the higher dose (10 mg/kg bw) excreted about 35% of the administered dose as conjugates of 4′-hydroxy-3-phenoxy-4-fluorobenzoic acid, while females excreted about 5% more than males as the free metabolite. After repeated oral doses at 0.5 mg/kg bw for 14 days, 12–16% of labelled metabolite was found in the faeces as cyfluthrin, while < 1% was found when single oral doses were administered. After a single high dose at 10 mg/kg bw, 17–19% of the administered dose was recovered in the faeces as parent compound. The authors concluded that the metabolism of cyfluthrin is slightly dose-dependent (Ecker, 1983).

Three male rats received ^{14}C-labelled cyfluthrin (radiochemical purity, 98%) at a dose of 10 mg/kg bw and urine was collected over 0–8 h and 8–24 h after administration. As an initial analysis by thin-layer chromatography (TLC) revealed no difference in metabolites between animals, samples of urine were pooled by collection time for identification of metabolite structures. It was found that about 60% of the administered radioactivity was excreted in the urine in a conjugated form (glucuronide or sulfate). Most of this was identified as conjugates of 4′-hydroxy-3-phenoxy-4-fluorobenzoic acid (50%). A second major metabolite was identified after hydrochloric acid hydrolysis as a conjugate of 3-phenoxy-4-fluorohippuric acid (40%). These metabolites represented 33% and 27% of the administered radiolabel, respectively. A glycine conjugate constituted 2.5% of the conjugated metabolites (Ecker, 1982).

Groups of 10 male Wistar (TNO/W 74) albino rats were given cyfluthrin (purity, 95%) at a dose of 1, 5, 10, or 15 mg/kg bw by intraperitoneal (IP) injection, urine was collected for 3 days, for analysis of thiocyanate content. In addition, groups of 10 male and 10 female rats were exposed to cyfluthrin at a dose of 59, 93 or 180 µg/l for 4 h through inhalation. Excretion of thiocyanate in urine was monitored over 92 h.

It was found that after IP administration, 24–42% of the alpha-cyano group of cyfluthrin was excreted via the kidneys in the thiocyanate form. After exposure by inhalation, 4–6% (male) or 2–6% (female) of the alpha-cyano group of cyfluthrin was excreted via the kidneys in the thiocyanate form (Eben & Thyssen, 1981).

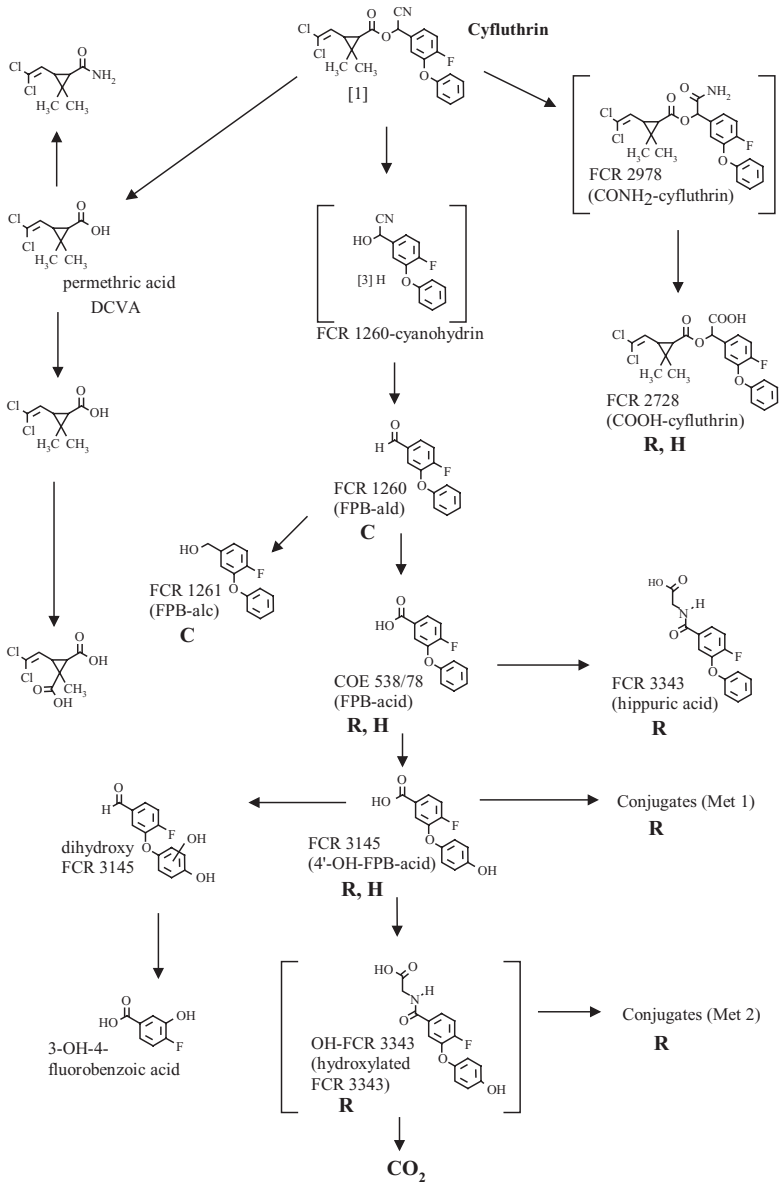
Cattle

A lactating Holstein dairy cow (*Bos Taurus*; bw, 484 kg) was given [^{14}C]-cyfluthrin (radiochemical purity, 98.5%) orally at a dose of 0.5 mg/kg bw after the evening milking each day for five consecutive days. Milk was collected in the morning and evening throughout the study. The cow was killed 1 day after the last dose, and blood, major organs, fatty tissue, and muscle were analysed for radiolabel.

The maximum concentration of radioactivity in the milk was 0.079 mg/l expressed as cyfluthrin equivalents 3 days after initial dosing; the level then declined steadily. Of the radiolabel found in milk, 98% was parent compound. The highest concentrations of residue were found in the liver (0.622 mg/kg), fat (0.195 mg/kg), and kidney (0.188 mg/kg); brain, skeletal muscle, and myocardium had the lowest concentrations, ranging from 0.015 to 0.040 mg/kg. Most of the radiolabel recovered in tissues was parent compound. Heart and kidney also contained a metabolite identified as 4-fluoro-3-phenoxybenzenemethanol, and liver also contained 4-fluoro-3-phenoxybenzaldehyde (Shaw et al., 1983).

The proposed metabolic pathway of cyfluthrin in rats is shown in Figure 2.

Figure 2. Proposed metabolic pathway of cyfluthrin in rats



2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with cyfluthrin are summarized in Table 2.

The observed clinical signs (increased salivation, uncoordinated movements, increased activity and vocalization, reduced, laboured breathing, apathy, straddle-legged gait (mostly in the rear legs), and reduced sensitivity to external stimuli) were typical of this class of pyrethroids.

Table 2. Results of studies of acute toxicity with cyfluthrin

Species	Strain	Sex	Route	Formulation	Purity (%)	LD_{50} (mg/kgbw)	LC_{50} (mg/l air)	Reference
Mouse	NMRI	Male Female	Oral[b]	PEG 400	83.6	291 609	—	Flucke & Thyssen (1980)
Mouse	NR	Female	Oral[b]	Cremophor EL/DW	NR	< 100	—	Heimann (1982a)
Rat	Wistar	Male Female	Oral	PEG 400	83.6	869 1271	—	Flucke & Thyssen (1980)
Rat	Wistar	Male Female	Oral[b]	PEG 400	83.6	590 1189	—	Flucke & Thyssen (1980)
Rat	NR	Male	Oral[b]	Acetone/oil	NR	254	—	Heimann (1982a)
Rat	NR	Male	Oral[b]	DMSO	NR	396	—	Heimann (1982a)
Rat	NR	Male	Oral[b]	Cremophor EL/DW	NR	16.2	—	Heimann (1982a)
Rat	NR	Male	Oral[b]	*N*-methyl-pyrrolidone	NR	500–1000	—	Heimann (1982a)
Rat	NR	Male	Oral[b]	Isodecane	NR	505	—	Heimann (1983a)
Rat	NR	Male	Oral[b]	Xylene	NR	499	—	Heimann (1983b)
Rat	NR	Male	Oral[b]	PEG 400	NR	795	—	Heimann (1984b)
Rat	NR	Male Female	Oral[b]	Acetone/oil	93	155 160	—	Heimann (1987a)[a]
Rat	Wistar	Male	Oral[b]	Cremophor EL/DW	95.1	15	—	Kroetlinger (1994)[a]
Rabbit	NZW	Male	Oral[b]	PEG 400	83.6	> 1000	—	Flucke & Thyssen (1980)
Dog	Beagle	Male	Oral[b]	NS	95	> 100	—	Hoffmann (1981a)
Dog	Beagle	Male	Oral	PEG 400	83.6	> 100[c]	—	Flucke & Thyssen (1980)

Species	Strain	Sex	Route	Formulation	Purity (%)	LD_{50} (mg/kgbw)	LC_{50} (mg/l air)	Reference
Sheep	NR	Male & Female	Oral[b]	Water/tylose	NR	1000	—	Hoffmann (1981b)
Hen	White Leghorn	Female	Oral	PEG 400	84–94	5000	—	Thyssen et al. (1981)
Hen	White leghorn	Female	Oral	Cremophor EL/DW	NR	> 5000	—	Thyssen (1982)
Hen	White Leghorn	Female	Oral	Cremophor EL/DW	93.5	> 5000	—	Sacchsse & Zbinden (1985a)[a]
Hen	White Leghorn	Female	Oral	PEG 400	93.5	4500	—	Sachsse & Zbinden (1985b)[a]
Rat	Wistar	Male Female	IP	Concentrated active substance	83.6	66 104	—	Flucke & Thyssen (1980)
Rat	NR	Male Female	IP	Cremophor EL/DW	NR	20 24	—	Heimann (1982a)
Rat	NR	Male Female	IP	NR	NR	34 94	—	Heimann (1982a)
Mouse	NMRI	Male emale	SC	PEG 400	83.6	> 2500 > 2500	—	Flucke & Thyssen (1980)
Rat	Wistar	Male Female	Dermal (24-h)	Cremophor EL/DW	83.6	> 5000 > 5000	—	Flucke & Thyssen (1980)
Rat	NR	Male Female	Dermal (24-h)	PEG 400	NR	> 5000 > 5000	—	Heimann (1982a)
Rat	NR	Male Female	Dermal (24-h)	NaCl	NR	> 5000 > 5000	—	Heimann (1982a)
Mouse	NMRI	Male Female	Inhalation (4-h)	Ethanol/PEG 400	93.9	—	± 0.141	Pauluhn (1989a)[a]
Rat	Wistar	Male & Female	Inhalation (1-h)	Ethanol/PEG 400	83.6	—	> 1.089	Flucke & Thyssen (1980)
Rat	Wistar	Male & Female	Inhalation (4-h)	Ethanol/PEG 400	83.6	—	> 0.469– 0.592	Flucke & Thyssen (1980)
Rat	NR	Male & Female	Inhalation (4-h)	Ethanol/PEG 400	*cis : trans* 55 : 45	—	0.180– 0.326	Flucke & Thyssen (1981)
Rat	Wistar	Male & Female	Inhalation	Ethanol/PEG 400	93	—	0.405	Pauluhn (1987a)[a]
Rat	Crj:CD	Male Female	Inhalation (4-h)	Ethanol/PEG 400	95	—	1.010 1.020	Watanabe et al. (1984)
Rat	NR	Male Female	Inhalation (4-h)	Cremophor EL/DW	95	—	> 0.735 0.200– 0.735	Pauluhn & Thyssen (1982)

Species	Strain	Sex	Route	Formulation	Purity (%)	LD_{50} (mg/kgbw)	LC_{50} (mg/l air)	Reference
Rat	NR	Male Female	Inhalation (4-h)	DMSO/PEG 400	95	—	0.575 0.490	Pauluhn & Thyssen, (1982)
Rat	Wistar	Male & Female	Inhalation (5 × 6 h)	Ethanol/PEG 400	83.6	—	0.047– 0.196	Flucke & Thyssen (1980)
Hen	NR	Female	Inhalation (4-h)	Ethanol/PEG 400; Cremophor EL/DW	NR	—	> 0.596	Pauluhn & Kaliner (1983)

DMSO, dimethyl sulfoxide; DW, distilled water; IP, intraperitoneal; NR, not reported; NZW, New Zealand White; SC, subcutaneous.

[a] Statements of adherence to quality assurance and good laboratory practice were included.

[b] Fasted animals.

[c] In the study in dogs, vomiting occurred at doses of 50 mg/kg bw and above.

(a) Dermal irritation

In a study of dermal irritation in NZW rabbits, performed in compliance with the United States Department of Agriculture guidelines (1973), six animals were exposed to cyfluthrin (purity, 83.6%) for 24 h on the intact and abraded skin. Irritation was scored at 24 h and 72 h.

No skin irritation was observed (Flucke & Thyssen, 1980).

In a study of dermal irritation in six female rabbits, 0.1 ml of cyfluthrin (purity, 95%) was applied under occlusion for 24 h on the intact or abraded skin of the inner ear. Irritation was scored at 24 h and 72 h.

No skin irritation was observed (Iyatomi et al., 1982).

(b) Ocular irritation

In a study of ocular irritation, performed in compliance with United States Department of Agriculture guidelines (1973), the eyes of New Zealand White rabbits were exposed to cyfluthrin (purity, 83.6%) for 5 min (five rabbits) or 24 h (three rabbits). The eyes were examined at 1 h and at 1, 2, 3 and 7 days.

Cyfluthrin induced moderate to severe redness and mild to moderate chemosis of the conjunctivae. The effects had almost completely resolved within 72 h (Flucke & Thyssen, 1980).

In a study of ocular irritation in female rabbits, 12 animals received 0.1 ml of cyfluthrin (purity, 95%) in the conjunctival sac of the left eye. The right eye served as control. In six of the animals, the eyes were rinsed after 30 s with 100 ml of tepid water. Eyes were examined at 1, 3, 6, 24, 48, 72 and 168 h.

Redness and chemosis of the conjunctiva was seen regardless of rinsing of the eye. The effects had almost completely reversed within 3 days. It was concluded that cyfluthrin has a minimal irritating effect on the eye (Iyatomi et al., 1982).

(c) Dermal sensitization

In a study of dermal sensitization, using the method reported by Draize, cyfluthrin (purity not reported) was tested using 15 male Pirbright White guinea-pigs. The control group consisted of 15 animals. The treatment regime involved induction of sensitization by 10 intracutaneous injections of

cyfluthrin (0.1% v/v) at a dose of 0.05 mg on day 1, 0.1 mg for the next nine treatments (three per week) and, after an interval of 14 days, 0.05 mg (challenge injection).

No signs of skin sensitization induced by cyfluthrin were observed (Mihail, 1981a).

In a study of dermal sensitization, conducted according to the Magnusson & Kligman method, cyfluthrin (purity not reported) was tested on 15 male Pirbright White W58 guinea-pigs. The control group consisted of 15 animals. In the induction phase, the animals received cyfluthrin by intradermal injection (1%) on day 1 followed by a topical administration (25% v/v) after 1 week and challenge by topical administration (25%) 3 weeks after the intracutaneous injection. The vehicle was PEG 400.

No signs of skin sensitization induced by cyfluthrin were observed (Mihail, 1981b).

The skin sensitization potential of cyfluthrin (purity, 96.2%) was tested in 20 male Hsd/Win:DH guinea-pigs, in a study conducted according to OECD guideline 406 using the Magnusson & Kligman maximization test. Ten animals served as controls. In the induction phase, the animals received cyfluthrin by intradermal injection (5%) on day 1 followed by a topical administration (50% w/v) after 1 week and challenge by topical administration (50% and 25%) 3 weeks after the intracutaneous injection. The vehicle was PEG 400. Statements of adherence to GLP and quality assurance (QA) were included.

After challenge with 50% cyfluthrin formulation, 5% of the animals showed reddening of the skin. No skin reactions were observed after challenge with 25% solution of cyfluthrin (Vohr, 1994a).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Groups of 18 male and 18 female mice (strain unknown) received diets containing cyfluthrin (purity unknown) at a concentration of 0, 300, 1000 or 3000 ppm (equal to 0, 43.1, 136.3 and 406.9 mg/kg bw per day for males and 0, 50.4, 164.5 and 432.8 mg/kg bw per day for females). After 1 month, 12 males and 12 females in each group were killed. The remaining animals were allowed to recover for 4 weeks. Animals were observed for clinical signs at 6 h and daily after start of the treatment. Body weight, food and water consumption were measured weekly. Haematology and clinical chemistry were performed at weeks 4 and 8. At weeks 4 or 8, the mice were killed, organs were weighed and the animals were examined macroscopically and histologically.

In the group receiving the highest dose, mice developed ataxia, salivation, and/or emaciation, and one female died. Body-weight was reduced at 3000 ppm (15–21%). A slight reduction (8%) in body weight, observed only at week 3 in females receiving the intermediate dose was considered not toxicologically relevant. Slight increases in alkaline phosphatase activity (maximally 35%) and blood urea nitrogen (BUN) (maximally 12%) at 1000 and 3000 ppm were considered not biologically significant. In males and females at 3000 ppm, macroscopy at 4 weeks revealed dark-red livers in some mice, and increased relative weights of the submaxillary glands (17–20%) and kidneys (13%). Males at 3000 ppm also had significantly increased relative liver weights (25%). Histopathological examination revealed minimally increased chromatin in the nuclei of hepatocytes of males at 1000 ppm or more and in females at the highest dose. Mice at 1000 ppm or more had cytoplasmic swelling of the glandular epithelium of the submaxillary glands. All findings disappeared within the 4-week recovery period. On the basis of the histological changes in the liver (males only) and the submaxillary glands, the NOAEL was 300 ppm, equal to 43.1 mg/kg bw per day (Watanabe et al., 1982b).

Rat

Groups of 20 male and 20 female Wistar rats were given technical cyfluthrin (purity, approximately 85%) at a dose of 0, 5, 20, or 40 (80) mg/kg bw per day by gavage for 28 consecutive days. PEG 400 served as vehicle. The highest dose of 80 mg/kg bw per day was administered during weeks 1 and 3, while a dose of 40 mg/kg bw per day was given during weeks 2 and 4. The highest dose was lowered after the first week due to toxicity in treated males; however, as these symptoms disappeared after 1 week at the lower dose, the highest dose was again raised in week 3. However, owing to severe symptoms of toxicity, the dose had to be lowered again in week 4. A group of 10 males and 10 females from each group was maintained on control diet without additional treatment for 6 weeks in order to assess recovery. Rats were observed daily for clinical signs and mortality and body weights were recorded weekly. Haematology, clinical chemistry and urine analysis were conducted for five males and five females at each dose at the end of the 28-day treatment period and at the end of the 6-week recovery period. At the end of the treatment period, half of the surviving rats in each treatment group were killed, while the remaining animals were killed after the 6-week recovery period. Animals were examined macroscopically and histologically and selected organs were weighed.

In the group receiving the highest dose, six males and one female died. At this dose, rats displayed apathy, ruffled coat, dyspnoea, salivation, hyperkinesis, ataxia, and uncoordinated movements. The symptoms were described as being most severe during weeks 1 and 3 (when a highest dose of 80 mg/kg bw per day was administered), and minimal during weeks 2 and 4 (when the highest dose was lowered to 40 mg/kg bw per day). Body weight was decreased by about 10% in males at the highest dose, but not in females at this dose. Clinical chemistry demonstrated slightly increased activity (20% and 68% in males and females, respectively) of alanine aminotransferase (ALT) in the group at the highest dose. No effects on haematology and urine analysis were found. At necropsy in rats at the highest dose, increased absolute (15% and 19% in males and females, respectively) and relative (38% and 19% in males and females, respectively) adrenal weights were noted. In females at this dose, absolute and relative liver weight was increased by 24%. No toxicologically relevant macroscopic or histological changes were observed. All effects related to treatment with cyfluthrin returned to normal during the 6-week recovery period. On the basis of effects on mortality, clinical signs, body weight (males only) and effects on absolute and relative adrenal and liver weights, the NOAEL was 20 mg/kg bw per day (Flucke & Schilde, 1980).

Groups of 18 male and 18 female rats (strain unspecified) received diets containing cyfluthrin (purity unknown) at a concentration of 0, 100, 300, or 1000 ppm (equal to 0, 8.3, 24.7 and 78.9 mg/kg bw per day for males and 0, 8.4, 25.2 and 77.9 mg/kg bw per day for females) for 4 weeks, when 12 males and 12 females of each group were sacrificed. Six males and six females per group were allowed to recover for 4 weeks. Animals were observed for clinical signs at 6 h and daily after start of the treatment. Body weight, food and water consumption were measured weekly. Urine analysis, haematology and clinical chemistry were performed at week 4 and 8. At week 4 or week 8 animals were killed, organs were weighed and the animals were examined macroscopically and histologically.

No mortality was observed. Clinical signs in rats at the highest dose consisted of straddled-gait, salivation and/or nervousness during treatment. A decrease in body weight (up to 19%), food intake (up to 35%), and water consumption (up to 37%) was observed at 1000 ppm. Urine analysis indicated a higher incidence of urobilinogen and ketone bodies in rats at 1000 ppm. Haematology demonstrated a decrease in erythrocyte count, erythrocyte volume fraction, and haemoglobin content at 1000 ppm. Blood glucose concentrations were significantly decreased in both sexes at 1000 ppm (17–18%) and in males at 300 ppm (12%). No effects were seen on macroscopic examination. Histopathology revealed minimal degeneration of single fibres of the sciatic nerve and cytoplasmic swelling of the

glandular epithelium of the submaxillary glands at the highest dose. All observed changes induced by treatment with cyfluthrin returned completely to normal during the 4-week recovery period, except for the effects on total protein and blood glucose, which showed a partial recovery. On the basis of the reduced blood glucose concentrations in male rats at 300 ppm, the NOAEL was 100 ppm, equal to 8.3 mg/kg bw per day (Watanabe et al., 1982a).

Groups of 30 male and 30 female Wistar rats were fed diets containing cyfluthrin (purity, 84.2%, 50% diluted in a premix with Wessalon S) at a concentration of 0, 30, 100, or 300 ppm (equal to 0, 2.2, 7.4 and 22.5 mg/kg bw per day in males and 0, 2.7, 8.8 and 28.0 mg/kg bw per day in females) for 3 months. Rats were examined daily for mortality and clinical signs. Body weights and food consumption were recorded weekly. Clinical chemistry was conducted at months 1 and 3. In addition, five males and five females per group were killed after weeks 1 and 4 for interim examinations, including assays for induction of microsomal enzymes (*N*- and *O*-demethylase and cytochrome P450 content). At the end of the treatment period, the remaining rats were killed, macroscopically and histologically examined and organs were weighed.

No effects on mortality, clinical signs, body weight, food consumption, haematology, serum chemistry, urine analysis, macroscopy, histology and organ weights were observed. At week 1, a slight, dose-independent increase in *N*-demethylase activity was seen in all treated males and in *O*-demethylase activity in females at 100 and 300 ppm; the activity of enzymes of the cytochrome P450 system was significantly increased only in males at 300 ppm at this time. By 3 months, no differences were seen in the activities of the microsomal enzymes in any treated group.

On the basis of the absence of toxicologically relevant effects at 3 months, the NOAEL was 300 ppm, equal to 22.5 mg/kg bw per day (Loser & Schilde, 1980).

In a 3-month feeding study, groups of 28 male and 28 female Sprague-Dawley rats were fed diets containing technical cyfluthrin (purity, 95%, incorporated into the powdered diet on a 0.4% maximum clay carrier) at a concentration of 0, 100, 300, or 1000 ppm (equal to 0, 6.2, 19.0 and 61.0 mg/kg bw per day for males and 0, 7.3, 21.2 and 68.5 mg/kg bw per day for females). Twenty rats from each group were killed after 3 months of treatment, and the remaining eight rats from each group were maintained on control diets for one additional month. Rats were observed daily for clinical signs. Body weights were recorded weekly. Food consumption was measured three times per week. Clinical chemistry and urine analysis studies were conducted after 3 months of treatment, or after the 1-month recovery period. At termination, organs were weighed and the rats were examined macroscopically and histologically.

At the highest dose, rats displayed abnormal gait and excess salivation during the first weeks of treatment. The number of rats that displayed these symptoms decreased over the treatment period, so that by the end of 3 months, no abnormal symptoms were noted. Mortality rate was not affected by treatment. Body-weight gain and food consumption were reduced by about 16% in rats at the highest dose. In the females, but not the males of the recovery group, a partial rebound of body weight was noted. Haematology or urine analysis parameters were not affected by treatment. The slight increases (maximally 16%) in aspartate aminotransferase (AST) and BUN values observed in the groups at the intermediate and highest doses were considered to be not biologically significant. ALT activity appeared to be decreased in treated animals. Blood glucose concentrations were depressed in males at 300 (−15%) and 1000 ppm (−29%) and in females at 1000 ppm (−17%). No significant changes in serum chemistry values were apparent at the end of the recovery period. The observed reductions in absolute organ weight at termination were considered to be related to the reduced body weight. No treatment-related macroscopic changes were noted. Histological examinations revealed an increased incidence of single-fibre degeneration of the sciatic nerve in 5 out of 20 males at the highest dose and 3 out of 20 females at the highest dose, when compared with an incidence of zero in the control

group and in the groups at the lowest and intermediate doses. In the 1-month recovery group, the incidence of this finding was one out of eight in males at the highest dose, and zero out of eight in all other groups.

On the basis of the reductions in blood glucose in males at the intermediate dose, the NOAEL was 100 ppm, equal to 6.2 mg/kg bw per day (Oikawa & Iyatomi, 1983).

Dogs

In a 6-month study, groups of six male and six female beagle dogs were fed diets containing technical cyfluthrin (purity, 90.8%) at a concentration of 0, 65, 200, or 600 ppm, equal to 0, 2.0, 6.5, and 19.9 mg/kg bw per day, respectively. Food consumption was measured daily. Body weights were determined weekly. Dogs were examined daily for clinical signs. Examinations of reflexes, pulse and body temperature, and haematology, clinical chemistry, urine analysis and ophthalmoscopy were performed before treatment initiation, and after 4, 7, 13, and 26 weeks. At termination, dogs were examined macroscopically and histologically, and organs were weighed.

No deaths occurred and reflexes and body temperature were not affected by treatment. At the highest dose, five out of six males and three out of six females showed signs of uncoordinated, stiff gait of the hindlimbs, persisting for several hours, on several occasions after week 21. Vomiting after dosing occurred slightly more often in animals at the highest dose than in the controls. Diarrhoea occurred in all groups, including the controls, but much more frequently in dogs at the highest dose, from the start of treatment. Body-weight gain was decreased in males at the intermediate dose but not at at the highest dose. Therefore this reduction in body-weight gain in males was considered to be not related to treatment. In females at the highest dose, a reduction in body-weight gain was observed that may have been related to treatment. No effects were found on ophthalmoscopic, haematological, clinical chemistry, or urinary parameters or on organ weights. Macroscopical and histological examination revealed no treatment-related changes. No lesions of the nervous system were observed. On the basis of the clinical signs in both sexes and reduced body-weight gain in females at the highest dose, the NOAEL was 200 ppm, equal to 6.5 mg/kg bw per day (Hoffmann & Kaliner, 1981).

Groups of six male and six female beagle dogs were fed diets containing technical cyfluthrin (purity, 90.8%) at a concentration of 0, 40, 160, or 640 ppm, equal to about 0, 1.4, 5.1, and 22.9 mg/kg bw per day for both sexes, for 12 months. Food consumption and clinical signs were assessed daily. Body weights were determined weekly. A complete physical examination including reflexes and body temperature, haematology, clinical chemistry and urine analysis were carried out before treatment initiation, and after 6, 13, 26, 39 and 52 weeks of treatment. Ophthalmoscopy was conducted before treatment initiation, and after 5, 13, 29, 39 and 52 weeks of treatment. At termination, dogs were necropsied and organs and tissues from dogs in the control group and at the highest dose were histologically examined.

During weeks 36–37, two males at the highest dose displayed clumsy, stiff movements of the hindquarters and a reluctance to move. Males at the highest dose had higher incidences of vomiting and soft, pasty faeces, and decreased body weights (−10%). Food and water consumption, physical examination, body temperature, ophthalmoscopy, haematology, clinical chemistry or urine analysis parameters were not affected. No treatment-related macroscopic or histological changes (including the sciatic nerve) were noted. The absolute and relative weight of spleen was increased by about 70% in females at the highest dose.

On the basis of decreased body weight, soft faeces and disturbances in the gait in males and the increased spleen weight in females at the highest dose, the NOAEL was 160 ppm, equal to 5.1 mg/kg per day (Hoffmann & Schilde, 1983).

In a study performed according to OECD guideline 453, groups of four male and four female beagle dogs were given diets containing cyfluthrin (purity, 94.8–95.1%) at a concentration of 0, 50, 100, 360 and 640 ppm (equal to 0, 1.4, 2.4, 10.6 and 15.5 mg/kg bw per day for males and 0, 1.5, 3.6, 10.7, and 18.0 mg/kg bw per day for females) for 12 months. Since the group at the highest dose began to demonstrate severe neurological symptoms in the first few weeks of the study, the highest dose was reduced to 500 ppm, beginning on week 8 and for the remainder of the study. Food consumption and clinical signs were assessed daily. Body weights were determined weekly. A detailed neurological examination was performed before testing, at 6 months and at termination. An electrocardiogram (ECG) and blood pressure were measured before the start of the treatment and at termination. Haematology, clinical chemistry (including plasma cholinesterase activity) and urine analysis were carried out before treatment initiation, and after 3, 6, 9 and 12 months. At termination, organs were weighed and the dogs were examined macroscopically and histologically. Statements of adherence to GLP and QA were included.

Two dogs in the control group (one male, one female) died from asymptomatic idiopathic epilepsy. One female at the highest dose was killed for welfare reasons when it developed a severe neurological condition that was considered to be treatment-related. Neurological examination at 6 and 12 months demonstrated dose-dependent gait abnormalities (hypermetria, reluctance to walk) and postural reaction deficits (abnormal head placement during wheel barrowing and abnormal foot placement during backward stepping, abnormal foot placement during lateral hopping, abnormal hemistanding posture) in the groups at 360 and 640/500 ppm. One female at the highest dose displayed ptosis, deficits in direct and indirect pupillary responses and protrusion of the membrane nictitans. Food consumption was not affected. At the highest dose, body weight was decreased by 17% in males. Reductions in female body weights in all treatment groups could be attributed to the death of a small female in the control group. ECG, blood pressure, clinical chemistry, plasma cholinesterase, haematology or urine analysis were not affected. Reductions of absolute organ weight in the treatment groups were considered to be secondary to the reduced body weights. No treatment-related macroscopic or histological changes were found.

On the basis of the neurological effects, the NOAEL was 100 ppm, equal to 2.4 mg/kg bw per day (Jones & Hastings, 1997).

(b) Dermal administration

Rats

In a 7-day study from published literature, groups of five male albino rats received daily dermal applications of a 40% formulation of cyfluthrin at a dose (active ingredient) of 0, 80, 160, 240 or 320 mg/kg bw per day under occlusion. The animals were killed 24 h after the last application. An additional group of five males and five females received cyfluthrin as a daily dermal dose at 100 mg/kg bw per day for 7 days, and was subsequently allowed to recover for 20 days. Animals were observed for clinical signs. At the end of the treatment period, blood was sampled for determination of alkaline phosphatase (AP), ALT, AST, total bilirubin, total protein, albumin, β-globulin and acetylcholinesterase activity.

All animals at the two higher doses died within 4 days after initiation of the treatment. At all doses, increases in AP, ALT, AST, total bilirubin and β-globulin were observed. Total protein, albumin, total globulin and acetylcholinesterase activity were reduced. Apart from the bilirubin concentrations, all parameters returned to normal within 15 days after cessation of treatment (El-Elaimy, 1986).

In a study of dermal toxicity, performed according to OECD guideline 411, groups of eight male and eight female Sprague-Dawley rats were treated dermally for 21 days with technical-grade

cyfluthrin (purity, 95.5–95.9%) at a dose of 0, 100, 340 or 1000 mg/kg bw per day. Doses were administered with a moistened pad to the shaven skin on the back of the animal. An additional eight males and eight females were included with the control group and the group at the highest dose and were allowed to recover for 2 weeks after cessation of the treatment. Animals were checked daily for mortality and clinical signs. Body weight and food consumption were measured weekly. Ophthalmoscopy was performed before dosing and at the end of the treatment period. At termination, blood was sampled for haematology and clinical chemistry, organs were weighed and macroscopy and histology was performed. Statements of adherence to GLP and QA were included.

No mortality or ocular abnormalities were observed. Body weight was not affected. Food consumption at the highest dose was significantly reduced during the first week of treatment. At the highest dose, a red discharge from the nose and urine stains were observed. At the intermediate and highest dose, scabbing at the application site was found. Clinical chemistry or haematology parameters and organ weights were not affected by treatment. At the application site, macroscopy revealed crusty, discolored and/or raised zones, and histological examination showed extensive areas of moderate to marked ulceration with bordering epidermis thickened by acanthosis and hyperkeratosis. There was inflammatory cell infiltration in the exposed dermis underlying the ulceration at the intermediate and highest dose. An accompanying minimal to slight dermal fibrosis in two females at 1000 mg/kg bw per day was also noted. After a 2-week recovery period, the local skin lesions partially recovered. On the basis of the effects on food consumption during the first week and clinical signs, the NOAEL for systemic effects was 340 mg/kg bw per day (Warren et al., 1996).

Rabbits

In a 3-week study, groups of six male and six female New Zealand White rabbits were exposed to technical cyfluthrin (purity, 83.5%; dissolved in PEG 400) at a dose of 0, 50 or 250 mg/kg bw per day by dermal application. The skin of three males and three females in each group was abraded before application. Animals were exposed for 6 h each day for 5 days per week for 3 weeks. At the end of each 6-h application, the treated skin was washed. Rats were observed daily for changes in appearance or behaviour, and body weights were recorded weekly. The application site was examined for signs of skin irritation. Studies of clinical chemistry and urine analysis were conducted on all rabbits before the initiation of treatment and at study termination. At termination, the rabbits in each group were examined for gross lesions and changes in organ weights. A selected number of tissues from rabbits in the control group and at the highest dose, along with testes from rabbits at the intermediate dose, were examined histologically.

Examination of appearance, behaviour, body weight, haematology, clinical chemistry, urine analysis and organ weights showed no treatment-related effects. Gross and histopathological examination showed no effects attributable to treatment with cyfluthrin. The NOAEL was 250 mg/kg bw per day (Flucke & Vogel, 1980).

(c) Inhalation

In a short-term study of exposure by inhalation, two experiments were performed. In the first experiment, groups of 10 male and 10 female Wistar rats were exposed (head/nose only) to aerosols (90% of particles < 3 μm) of technical cyfluthrin (purity, 83.1–85.3%) at actual concentrations of 0, 2.3, 11.5 and 69.6 μg/l for 6 h each day for 5 days per week for 3 weeks (a total of 15 exposures). The solvent used in the generating system (and the control atmosphere) was a 1 : 1 mixture of ethanol and PEG 400. Rats were observed daily for clinical signs. Body weights were recorded weekly. Rectal body temperatures were measured before and after exposures 1, 5, 10 and 15. Clinical chemistry and urine analysis were performed on five males and five females at each dose after the last exposure. At

termination, the rats were killed, examined macroscopically and organs were weighed. Five males and five females from each group were examined microscopically.

A second experiment was performed with the same design as the first experiment except that clinical chemistry was not performed. In this experiment, rats were exposed to cyfluthrin at actual concentrations of 0, 0.4, 1.4 or 10.5 µg/l.

In the first experiment, animals at the intermediate and highest dose displayed signs of ungroomed coat, abnormal gait, and increased salivation. One female at the highest dose died. Body-weight gain was affected in all treatment groups, as treated rats either lost body weight or failed to gain weight during the treatment period. Rats in the control group showed normal weight gains. As compared to values measured before treatment, body temperatures were consistently lowered after treatment. Haematology, clinical chemistry and urine analysis studies did not reveal any significant effects. No evidence of induction of liver drug-metabolism enzymes was apparent. No macroscopic lesions and no toxicologically relevant effects on organ weight were noted. Histological examinations revealed treatment-related increases in inflammation of trachea and in the incidence of emphysema in rats at the highest dose. On the basis of the effects on body-weight gain in all treatment groups in this experiment, the lowest-observed-adverse-effect concentration (LOAEC) was 2.3 µg/l air.

In the second experiment, it was reported that behavioural changes (data not shown) were noted only in rats at the highest dose during the final week of treatment. Body-weight gain was decreased in rats at the highest dose, in particular during the first 2 weeks of treatment. At termination, no treatment-related gross lesions were noted. At the highest dose, absolute and relative spleen weight was slightly reduced (absolute, up to 18%; relative, up to 13%). Small reductions (≤ 6%) in absolute and relative liver weights in rats at the intermediate and highest dose were considered to be not toxicologically relevant. No treatment-related lesions were noted after histological examinations.

Taking the data from both experiments together, the no-observed-adverse-effect concentration (NOAEC) was 1.4 µg/l air, on the basis of the retarded body-weight gain at 2.3 µg/l (Thyssen & Mohr, 1980).

In a short-term study of exposure by inhalation, performed in accordance with OECD 412, groups of 10 male and 10 female Wistar rats were exposed to cyfluthrin (purity, 93.8%) for 4 weeks (6 h each day, 5 days per week) at actual concentrations of 0, 0.44, 6.0, and 46.6 mg/m³ air (head/nose exposure). Mortality, clinical signs and body weights were assessed daily. Reflexes (cornea, pinna, light, startle, righting, myotactic), and lung function were assessed weekly. Rectal temperature was measured twice per week, 5–20 min after the end of exposure. Ophthalmoscopy, clinical chemistry, haematology and urine pH tests were performed on five males and five females in each group at the end of the study. In an additional five males and five females, arterial blood gases and acid-base status was determined. At termination, the rats were necropsied, organs were weighed and histologically examined. Statements of adherence to GLP and QA were included.

At the highest concentration, mild to moderate clinical signs of a non-specific nature (ruffled coat, hyperactivity, bradypnoea) were observed after the end of exposure. At concentrations of 6 mg/m³ and higher, a transient reflex bradypnoea was observed. At the same concentrations, a slight, transient reduction in body temperature, as well as a reduction in leukocyte counts, reduced absolute (up to 38%) and relative (up to 28%) thymus weights and (in the males only) decreased body weight (10%) were found. Slight acidification of the urine, observed at the highest dose was considered to be secondary to the reflexive bradypnoea. No other treatment-related effects were observed.

On the basis of transitory bradypnoea, slight transitory reduction of body temperature, leukocyte count, thymus weight (in both sexes), and growth retardation (only in males) the NOAEC was 0.44 mg/m³ (0.044 µg/l air) (Pauluhn, 1989b).

In a short-term study of exposure by inhalation, groups of 10 male and 10 female Wistar rats were exposed to cyfluthrin (purity, 94.9%) in aerosols (> 85% of particles were < 5µm) at actual concentrations of 0, 0.09, 0.71 and 4.52 µg/l for 6 h each day for 5 days per week for 13 weeks (a total of 63 exposures). The solvent used was a 1 : 1 mixture of ethanol and PEG 400. Rats were observed daily for clinical signs. Body weights were recorded weekly. Clinical chemistry and urine analysis studies were conducted on all rats after 6 and 13 weeks of treatment. At termination, the rats were killed, examined macroscopically and histologically and organs were weighed. Statements of adherence to GLP and QA were included.

Signs of non-specific behavioural disturbances were observed in all males at the highest dose and in all females at the intermediate and highest dose. Body weight (8–15%) was reduced at concentrations of 0.71 mg/m³ air and greater in the males, but only at 0.71 mg/m³ air in the females. No effects of treatment on haematology, clinical chemistry or urine analysis parameters were observed. Small (maximally 10%) but statistically significant reductions in relative liver weights were observed in rats at the intermediate and highest dose. Measurements of *N*-demethylase, *O*-demethylase and cytochrome P450 activities yielded no evidence of enzyme induction. Macroscopic and histological examinations did not reveal any evidence of specific organ damage.

On the basis of behavioural changes and reduced body weight, the NOAEC was 0.09 µg/l air (Pauluhn & Mohr, 1984).

Four male Sprague-Dawley rats with permanently implanted intra-arterial catheters were exposed nose-only for 4 h to aerosols containing cyfluthrin (purity, 96.2%) at analytical concentrations of 13.2 µg/l air. Body temperature, blood gas concentrations and blood pH were assessed. Statements of adherence to QA and GLP were included.

A distinct hypothermia developed during the 4-h exposure period. Furthermore, decreases in arterial partial pressure of carbon dioxide and a rise in arterial blood pH were demonstrated. These results corroborated the hypothesis that the reflex bradypnoea, which in turn has been induced by sensory irritation, induces secondary hypothermia and respiratory alkalosis. The study author suggested that these effects influenced embryonic development and cause dysmorphogenesis as observed by Holzum (1993) (Pauluhn, 1992).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female CF1/W74 mice received diets containing cyfluthrin (purity, 90.8%) at a concentration of 0, 50, 200, or 800 ppm (equal to 0, 11.6, 45.8 and 194.5 mg/kg bw per day in males and 0, 15.3, 63.0 and 259.9 mg/kg bw per day in females) in the form of a 50% premix with Wessalon S for 23 months. Animals were examined daily for mortality and clinical signs. Body weights and food consumption were determined weekly. Haematology, clinical chemistry and urine analysis were performed on 10 males and 10 females per group at 0, 6, 12, 18 and 23 months. The fluoride content of bones and teeth was determined for five males and five females in each group after 23 months of treatment. At termination, the remaining animals were examined macroscopically and histologically, and organs were weighed.

No treatment-related effects were observed on mortality, clinical signs, haematological parameters, fluoridation of bones or teeth, food intake, or water consumption. Body weight was reduced (up to 10% in males and 12% in females) at the highest dose, in particular during the second year of treatment. Plasma AP activity was increased in all treated males at 6, 12 and 18 months. However, at all doses the increases were small (36–74%, except for the measurements at 18 months in males at 50 ppm when the increase was 114%, and the measurement at 6 months in males at 800 ppm

when the increase was 232%), and the increases were not strictly dose-dependent. At termination, the highest levels of AP activity were found in males in the control group. In females, treatment with cyfluthrin had no observed effect on AP activity. Histological examination revealed no evidence of liver damage. ALT was slightly elevated in males at the highest dose after 6 and 18 months of treatment, but not after 12 months. No treatment-related macroscopic, histological or organ weight changes were seen. The incidences of non-neoplastic and neoplastic lesions were all within normal ranges.

On the basis of the effects on body weight, the NOAEL was 200 ppm, equal to 45.8 mg/kg bw per day (Suberg & Loser, 1983a).

Groups of 50 male and 50 female CD-1 mice were given diets containing technical cyfluthrin (purity, 93.9–95.1%) at a concentration of 0, 200, 750 or 1400/1600 (male/female) ppm (equal to 0, 31.9, 114.8 and 232.7 mg/kg bw per day for males and 0, 38.4, 140.6 and 309.7 mg/kg bw per day for females) for 2 years. Animals were examined daily for mortality and clinical signs. A detailed physical examination was performed weekly. Body weights and food consumption were recorded weekly. Ophthalmoscopy was performed before the start of treatment and at 12 and 24 months. Haematology was performed at 12 and 18 months on 10 males and 10 females per dose. At termination, animals were examined macroscopically and histologically and organs were weighed. Statements of adherence to GLP and QA were included.

Mortality was not affected by treatment. An increased incidence of rough coat was observed in both sexes at 1400/1600 ppm. At the highest dose, females displayed hunched back, and lesion redness and lesion scab, generally associated with the ear pinnae of one or both ears. Body weights were significantly reduced in males in the group at the highest dose (up to 10%) and in females at the intermediate and highest dose (10–20%). Food consumption and haematological parameters were not affected. Macroscopy revealed rough coat in males and females at 1400/1600 ppm, crusty zones of the skin of the ear in males at 750 ppm and males and females at 1400/1600 ppm, and wet/stained ventrum in males at 1400 ppm. Histological examinations revealed increased incidences of acanthosis, chronic active inflammation, inflammation (all types), ulcer, and debris of the skin of the ears of males at 750 ppm and males and females at 1400/1600 ppm. No evidence for a tumorigenic effect of cyfluthrin was found. Observed reductions in absolute organ weight were likely to be secondary to the reduced body weight, and were considered to be not toxicologically relevant.

On the basis of the reductions in body weight in the female mice and the macroscopic and histological effects in males, the NOAEL was 200 ppm, equal to 38.4 mg/kg bw per day (Wahle & Christenson, 1998).

Rats

Groups of 65 male and 65 female Wistar rats were fed diets containing technical cyfluthrin (purity, 90.8%) at a concentration of 0, 50, 150 or 450 ppm (equal to 0, 2.0, 6.2, and 19.2 mg/kg bw per day in males and 2.7, 8.2, and 25.5 mg/kg bw per day in females) for 2 years. Animals were examined daily for abnormalities of behaviour or appearance. Body weights were recorded weekly through study week 27, every other week from week 27 to week 74, and then weekly until study termination. Food consumption was measured weekly. Haematology, clinical chemistry and urine analysis were performed at 6, 12, 18, and 24 months on 10 males and 10 females per group. Serum protein electrophoresis was performed at 12 months. At day 7 of treatment, mixed function oxidase activities and liver cytochrome P450 content were assessed in five males and five females per group. After 12 and 24 months of treatment, the fluoride content of bones and teeth was determined.

Ten males and 10 females per group were killed after 12 months of treatment, of which five males and five females per group were perfused with 10% buffered formalin before gross examination

and dissection. At termination, animals were macroscopically and histologically examined and organs were weighed (except for animals perfused with formalin).

No treatment-related effects on mortality, clinical signs, food consumption, haematology or urine analysis were observed. Occasional changes in blood glucose, alpha-1-globulin and leukocyte count were considered to be not toxicologically relevant. Compared with controls, body weights were significantly decreased throughout the study by about 8–10% at the highest dose. The reduction in body-weight gain (about 5%) in males at the intermediate dose during the first year of treatment was considered to be not biologically significant. At day 7 of treatment, *N*-demethylase activity in the group at the highest dose was increased by 30% (males) and 70% (females). *O*-Demethylase activity and cytochrome P450 content were not affected. Fluoride content of bones of males at the intermediate and highest dose and females at the highest dose was increased at 24 months, but not at 12 months. Macroscopic and histological examination and organ weights revealed no toxicologically relevant effects. Treatment did not affect the total or specific incidence of tumours.

On the basis of decreases in body weight in the group at the highest dose, the NOAEL was 150 ppm, equal to 6.2 mg/kg bw per day (Suberg & Loser, 1983b).

Groups of 60–70 male and 60–70 female Fischer 344 rats were given diets containing technical cyfluthrin (purity, 93.9–95.1%) at a concentration of 0, 50, 225, or 450 ppm (equal to 0, 2.6, 11.6, and 22.8 mg/kg bw per day for males and 0, 3.3, 14.4, and 28.3 mg/kg bw per day for females) for 2 years. Animals were examined daily for mortality and clinical signs. A detailed physical examination was performed weekly. Body weights and food consumption were recorded weekly. Ophthalmoscopy was performed before the start of treatment and at 12 and 24 months. Haematology, clinical chemistry and urine analysis were performed at 3, 6, 12, 18, and 24 months on 20 males and 20 females per group. Twenty males and 20 females in the control group and the group at the highest dose and 10 males and 10 females at the lowest and intermediate doses were killed for interim examination after 12 months of treatment. At termination, animals were examined macroscopically and histologically and organs were weighed. Statements of adherence to GLP and QA were included.

Body weight was reduced by 11% in the group at the highest dose. The reduction in body-weight gain (about 6%) at the intermediate dose was considered to be not biologically significant. At the highest dose, an increased incidence of alopecia was observed. Clinical chemistry demonstrated a slight reduction in serum triglyceride concentration (and, to a lesser extent, serum cholesterol concentration) in males at the highest dose. Food consumption, survival, ophthalmology, haematology, and urine analysis were not affected by treatment. No treatment-related changes in macroscopic and histological parameters and no evidence for a tumorigenic effect of cyfluthrin were found. Observed reductions in absolute organ weight were considered to be secondary to the reduced body weight, and not toxicologically relevant.

On the basis of the reduced body weight and alopecia, the NOAEL was 225 ppm, equal to 11.6 mg/kg bw per day (Wahle & Christenson, 1997).

2.4 Genotoxicity

The results of tests for genotoxicity are summarized in Table 3. The Meeting concluded that cyfluthrin is unlikely to be genotoxic.

Table 3. Results of studies of genotoxicity with cyfluthrin

End-point	Test object	Concentration[a]	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium.* strains TA98, TA100, TA1535 and TA1537	20–24 000 µg/plate (± S9)	83.6	Negative	Herbold (1980a)
Reverse mutation	*S. typhimurium*. strains TA98, TA100, TA1535, TA1537 and TA1538	5–5 000 µg/plate (± S9)	95.0	Negative	Nagane et al. (1982)
Reverse mutation	*S. typhimurium*. strains TA98, TA100, TA1535, TA1537 and TA 1538	10–25 000 µg/plate (± S9)	95.0	Negative	Ohta & Moriya (1982)
Reverse mutation	*E. coli* WP*2urvA*	5–5 000 µg/plate (± S9)	95.0	Negative	Nagane et al. (1982)
Reverse mutation	*E. coli* WP*2urvA*	10–25 000 µg/plate (± S9)	95.0	Negative	Ohta & Moriya (1982)
Reverse mutation	*S. cerevisiae S138*	312.5–10 000 µg/ml (± S9)	95.0	Negative	Brusick (1982a,b)
Reverse mutation	*S. cerevisiae D7*	625–10 000 µg /ml (± S9)	95.0	Negative	Brusick (1982c)
Gene mutation	CHO K1-BH4 cells, *Hprt* locus	3–10 µg/ml (± S9)	94.7	Negative	Yang & Louie (1985)[b]
Chromosomal aberrations	Chinese hamster lung cells	$3.3 \times 10^{-5} – 3.3 \times 10^{-3}$ mol/l (± S9)	93.7	Negative	Sasaki et al. (1986)[b]
Chromosomal aberrations	Human lymphocytes	500–5 000 µl/ml (± S9)	95.1 –95.5	Negative	Herbold, (1988a)
Sister chromatid exchange	CHO cells	3–20 µg/ml (± S9)	Technical	Negative	Putnam (1985)[b]
DNA damage	*E. coli* pol A+ and pol A−	62.5–1 000 µg/plate (± S9)	95.0	Negative	Herbold (1981a)
DNA damage	*B. subtilis* H17, M45 rec−	200 µg/plate (+ S9)	95.0	Negative	Nagane et al. (1982)
DNA damage	*B. subtilis* H17, M45 rec−	100–10 000 µg/plate (+ S9)	95.0	Negative	Ohta & Moriya (1982)
Unscheduled DNA synthesis	Rat primary hepatocytes	17–5 000 µg/ml	94.7	Negative	Curren (1985)[b]
Mitotic gene conversion	*S. cerevisiae* D7	625–10 000 µg/ml (± S9)	95.0	Negative	Brusick (1982c)
Mitotic crossing over	*S. cerevisiae* D7	625–10 000 µg/ml (± S9)	95.0	Negative	Brusick (1982c)
In vivo					
Micronucleus formation	Mouse bone marrow	2 × 7.5 or 2 × 15 mg/kg bw (gavage, administration interval for 24 h)	83.6	Negative	Herbold (1980b)
Dominant lethal mutation	Mouse	30, 60 mg/kg bw, oral (gavage?) administration	83.6	Negative	Herbold (1981b)

[a] Positive and negative (solvent) controls were included in all studies.
[b] Statements of adherence to good laboratory practice and quality assurance were included.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study of reproductive toxicity, groups of 10 male and 20 femaleWistar (BOR:WISW) rats (age 5 to 6 weeks) received diets containing cyfluthrin (purity, 90.8%) at a concentration of 0, 50, 150, or 450 ppm (equal to 0, 3.8, 11.4 and 34.7 mg/kg bw per day for males and 0, 5.1, 14.0 and 46.9 mg/kg bw per day for females) as a 50% premix with Wessalon S. Treatment started 105 days before mating and continued throughout mating, gestation, and rearing of pups. Each male was mated with two females. The parameters measured included behaviour, body weight, food consumption, mortality, fertility, viability during gestation and lactation, development of the young, parental body-weight gain, and sex ratio. The study was conducted with two litters per generation. The parameters assessed included fertility, litter size, fetal birth weight, fetal variability, and parental weight gain. Macroscopy, histology and organ weight were examined for F_{2b} parental animals and F_{3b} pups.

Treatment had no effect on mortality and clinical signs. In the parental animals of the groups at the intermediate and highest dose, body-weight gain was decreased in a dose-dependent manner. Body-weight effects at the intermediate dose were mild (maximally 8% and 12% in males and females, respectively, only at a certain stage during the test period). No consistent treatment-related effects on fertility, sex ratio or the incidence of fetal abnormalities were observed. At the highest dose, birth weight was consistently reduced (7–10%). In the offspring of groups at the intermediate and highest dose, decreased body-weight gain during lactation and occasionally reductions in viability and lactation indices were observed. Adult reproductive parameters were not affected.

On the basis of the borderline reductions in body-weight gain at 150 ppm, the NOAEL for parental toxicity was 50 ppm, equal to 3.8 mg/kg bw per day. On the basis of the reduced body-weight gain during lactation and the occasional reductions in viability and lactation indices, the NOAEL for offspring toxicity was 50 ppm, equal to 5.1 mg/kg bw per day (based on the cyfluthrin intake of the dams). The NOAEL for reproductive toxicity was 450 ppm, equal to 34.7 mg/kg bw per day, i.e. the highest dose tested (Loser & Eiben, 1983).

In a two-generation study of reproductive toxicity, performed according to OECD 416, groups of 30 male and 30 female Sprague-Dawley CD rats received diets containing technical-grade cyfluthrin (purity, 94.6–96.2%) at a concentration of 0, 50, 125 or 400 ppm (equal to 0, 3, 9 and 29 mg/kg bw per day in males, 0, 4, 10 and 33 mg/kg bw per day in females during the premating period and gestation and 0, 7, 19 and 59 mg/kg bw per day for females during the lactation period). Animals were observed daily for mortality and clinical signs of toxicity. Body weights was determined weekly, and on days 0, 6, 13 and 20 of gestation and days 0, 4, 7, 14 and 21 of lactation. Food consumption was measured weekly, and twice per week during the first week of lactation. Estrous cycling, mating, fertility, duration of gestation, and litter size were assessed. In the offspring, sex ratio, pup viability, body-weight gain and clinical signs were determined. Macroscopy was performed on all adults and pups. Histopathological evaluation of reproductive organs, the pituitary, and gross lesions was performed on all parental and F_1 adults. Statements of adherence to QA and GLP were included.

Mortality was not affected by treatment. P and F_1 females at 400 ppm showed splaying of the hindlimbs during lactation. At 400 ppm, body weight was reduced (up to 14%) in the F_1 males, in the P females during gestation and in the P and F_1 females during lactation. The reduction in body

weight was accompanied by a decrease in food consumption. Adult reproductive parameters were not affected. In the F_1 and F_2 pups at 125 ppm and 400 ppm, coarse tremors were observed between day 5 and day 18 of lactation. No other treatment-related effects were observed in the pups. No treatment-related macroscopic and histological changes were observed in parental animals or offspring.

On the basis of effects on body weight and food consumption the NOAEL for parental toxicity was 125 ppm, equal to 9 mg/kg bw per day. On the basis of coarse tremors in the pups during lactation, the NOAEL for offspring toxicity was 50 ppm, equal to 7 mg/kg bw per day (based on the cyfluthrin intake of the dams during lactation). The NOAEL for reproductive effects was 400 ppm, equal to 29 mg/kg bw per day, i.e. the highest dose tested (Eigenberg & Elcock, 1996).

In a supplementary two-generation study of reproductive toxicity, performed according to OECD guideline 416, groups of 30 male and 30 female CD Sprague-Dawley rats received diets containing technical-grade cyfluthrin (purity, 95.5%–96.2%) at a concentration of 0, 25 or 50 ppm (equal to 0, 1.9 and 3.8 mg/kg bw per day in males, approximately 0, 2 and 4 mg/kg bw per day in females during the premating and gestation and 0, 4.1 and 8 mg/kg bw per day for females during lactation). Animals were observed daily for mortality and clinical signs of toxicity. Body weights was determined weekly, and on days 0, 6, 13 and 20 of gestation and days 0, 4, 7, 14 and 21 of lactation. Food consumption was measured weekly, and twice per week during the first week of lactation. Estrous cycling, mating, fertility, duration of gestation, and litter size were assessed. In the offspring, sex ratio, pup viability, body-weight gain and clinical signs were determined. Macroscopy was performed on all adults and pups. Histopathological evaluation of reproductive organs, the pituitary, and gross lesions was performed on all P and F_1 adults. Statements of adherence to QA and GLP were included.

No toxicologically relevant effects were observed on any of the parameters tested.

The NOAEL for reproductive effects, parental and offspring toxicity was 50 ppm, equal to 1.9 mg/kg bw per day, i.e. the highest dose tested (Eigenberg, 1997).

(b) Developmental toxicity

(i) Oral administration

Rats

Groups of 25 inseminated FB30 rats were treated with cyfluthrin (purity, about 85%) as daily oral doses at 0, 3, 10, and 30 mg/kg bw by gavage from day 6 to day 15 of gestation. Vehicle was PEG 400. Animals were observed for signs of toxicity and were weighed (time-points not specified). On day 20 of gestation, rats were killed and fetuses were examined for visceral and skeletal anomalies. The description of the methods and results was concise.

In the females receiving the intermediate or highest dose, a high-stepping gait was reported from the second week of treatment, and the females at the highest dose occasionally displayed ataxias and reduced motility (data not shown). Litter size, frequency of resorptions, and placental weights were not affected. Treatment did not affect mortality or maternal body-weight gain. No embryotoxic and/or teratogenic effects were found. Malformations were of a comparable type and frequency in all groups.

On the basis of the clinical signs, the NOAEL for maternal toxicity was 3 mg/kg bw per day. The NOAEL for embryo/fetotoxicity was 30 mg/kg bw per day, i.e. the highest dose tested (Schlüter, 1982).

Groups of 25 inseminated Wistar rats (KFM-HAN) were given technical-grade cyfluthrin (purity, 93.4%) as daily oral doses at 0, 1, 3, and 10 mg/kg bw by gavage from day 6 to day 15 of

gestation. The vehicle was 1% Cremophor EL/water. Rats were observed daily for mortality and signs of toxicity. Body weights were recorded daily, and food consumption was recorded on days 6, 11, 16 and 21 of gestation. On day 21 of gestation, dams were killed and ovaries and gravid uteri were removed by caesarean section for assessment of potential developmental effects. About one out of three of the live fetuses in each litter were fixed and examined for visceral changes, the remaining fetuses were examined for skeletal effects. Statements of adherence to GLP and QA were included.

No treatment-related signs or mortalities were observed. No treatment-related effect on body-weight gain (with or without gravid uterine weight) was observed. Necropsy revealed no effect of treatment on pre- or postimplantation loss, litter size, fetal viability or fetal body weight. Furthermore the incidences of malformations and fetal variations were not affected by treatment.

The NOAEL for maternal and embryo/fetotoxicity was 10 mg/kg bw per day, i.e. the highest dose tested (Becker, 1983).

Rabbits

Groups of 15 inseminated female Himalayan rabbits (strain CHBB:HM) were given technical-grade cyfluthrin (purity, 95.0%) at a dose of 0, 5, 15 or 45 mg/kg bw per day by gavage on days 6–18 of gestation. The vehicle was 0.5% Cremophor EL. Rabbits were observed for clinical signs of toxicity, and were weighed periodically during gestation. On day 29 of gestation, rabbits were killed and uteri were examined to assess the effects of treatment on fetal development. Fetuses were weighed and examined for visceral and skeletal abnormalities. The description of the study was concise.

No effects on mortality, behaviour, appearance, insemination rate, or maternal weight gain were observed. At the highest dose, two rabbits aborted on days 25 and 28 of gestation, and one rabbit completely resorbed its implants. Fetal body weight, placental weights or litter size were not affected by treatment. No effect of treatment on the incidences of visceral or skeletal abnormalities was observed. Arthrogryposis was seen in rabbits of all treatment groups. However, the incidence was not dose-dependent and this malformation was reported by the study authors to be one of the most common spontaneous malformations in this strain, with an incidence of 0.76%. Therefore, the occurrence of arthrogryposis was considered to be spontaneous.

On the basis of the two abortions and a single case of complete resorption, the NOAEL for maternal and embryo/fetotoxicity was 15 mg/kg bw per day (Roetz, 1983).

In a study of developmental toxicity that was performed according to OECD guideline 414, groups of 16 pregnant Chinchilla rabbits (Chb:CH hybrids, SPF) received cyfluthrin (purity, 96.0–96.1%) at a dose of 0, 20, 60, or 180 mg/kg bw per day by gavage on days 6–18 of gestation. Mortality, clinical signs and body weight were assessed daily. Food consumption was measured during days 0–6, 6–11, 11–15, 15–19, 19–24 and 24–28 of gestation. The dams were killed 28 days after mating and necropsied. Fetuses were examined for visceral and skeletal anomalies. Statements of adherence to GLP and QA were included.

Mortality and clinical signs were not affected by treatment. Food consumption and body weight were reduced in animals at 60 and 180 mg/kg bw per day, and were increased 24–28 days after mating. When compared with does in the control group, food consumption during the treatment period was decreased by 7, 26 and 40% in the groups at the lowest, intermediate and highest dose, respectively. When compared with their body weight on day 6, the does in the groups at the intermediate and highest dose showed a body-weight loss of 4% and 5%, respectively, during treatment, while in the control group and the group at the lowest dose, body-weight loss was only 0.5% during the same period. A dose-related, statistically significant increase in postimplantation loss, due to increased embryonic resorptions, was observed in rabbits at the intermediate and

highest dose. Necropsy revealed no significant alteration attributable to treatment. Sex ratio and fetal body weights were unaffected by treatment, and no significant alterations were found in fetal heads or brains. The observed skeletal alterations (delayed ossification of vertebrae, sternebrae, and ribs) were considered to be within the normal range for spontaneous occurrence. There was no indication of a teratogenic effect.

On the basis of the reduced food consumption and body-weight loss, the NOAEL for maternal toxicity was 20 mg/kg bw per day. On the basis of the increased postimplantation loss, the NOAEL for embryo/fetotoxicity was 20 mg/kg bw per day (Becker & Biedermann, 1992).

(ii) Exposure by inhalation

Rats

Two separate studies were performed in which groups of 30 inseminated Wistar rats (bor:WISW) received cyfluthrin (purity, 92.9%) at actual concentrations of 0, 1.1, 4.7, and 23.7 mg/m³ air (first experiment) or 0, 0.09, 0.25, and 0.59 mg/m³ air (second experiment) from day 6 to day 15 of gestation. Exposure was nose-only for 6 h per day under dynamic conditions. Oxygen substitution (30% oxygen) was carried out in a further group exposed at 4.16 mg/m³ air. Rats were observed daily for mortality and signs of toxicity. Body weights were recorded on days 0, 9, 12, 15 and 20 of gestation. On day 20 of gestation, dams were killed and ovaries and gravid uteri were removed by caesarean section for assessment of potential developmental effects. About one third of the live fetuses in each litter were fixed and examined for visceral changes, the remaining fetuses were examined for skeletal effects. Statements of adherence to GLP and QA were included.

No mortality was observed in the dams. At concentrations of 4.16 mg/m³ air and higher, reduced motility, ruffled unkempt fur, irritation of the visible mucous membranes and laboured breathing were observed. In the group at the highest concentration, these signs were already observed during the exposure period, while in the groups at 4.16 and 4.7 mg/m³ air they first appeared at the end of the exposure period. During the treatment period, body-weight gain of dams was significantly reduced at concentrations of 1.1 mg/m³ air and higher. At concentrations of 1.1 mg/m³ air and higher, significant reductions in fetal weights and a higher number of foetuses with retarded ossification were observed. In the group exposed at 4.16 µg/l air substituted with 30% oxygen, reductions in maternal body-weight gain, fetal weight and fetal ossification were much less pronounced. Therefore, the maternal and fetal effects were interpreted as signs of a non-specific retardation of embryonic development and were attributed to a maternal hypoxia induced by the treatment. No evidence of teratogenic effects was observed at any dose.

On the basis of the reduced body-weight gain, the NOAEC for maternal toxicity was 0.59 µg/l air. On the basis of the reduced body weight and the retarded ossification the NOAEC for embryo/fetotoxicity was 0.59 µg/l air. The NOAEC for teratogenicity was 23.7 µg/l air, i.e. the highest concentration tested. (Renhof & Pauluhn, 1988).

In a study of developmental toxicity performed according to OECD guideline 414, groups of 25 inseminated (Bor:WISW) Wistar rats were exposed nose-only to cyfluthrin (purity, 94.7–96.2%) at actual concentrations of 0, 0.46, 2.55 or 11.9 µg/l air, on days 6–15 of gestation. The vehicle was PEG 400. An additional group was exposed to cyfluthrin at 12.8 µg/l air, supplemented with 40% oxygen. Animals were checked daily for mortality and clinical signs. Body weight was assessed on day 0 of gestation, daily from day 6 to day 15 of gestation, and on day 20 of gestation. Food consumption was measured during days 0–6, 6–11, 11–16 and 16–20 of gestation. At day 20 of gestation, the dams were killed and necropsied, and fetuses were examined for visceral and skeletal abnormalities. For every group, a satellite group of five

females was exposed during days 6–13 of gestation for determination of mortality, clinical signs, body weight, food intake (daily on days 6–13 of gestation), lung function (day 6 of gestation only), reflexes and rectal temperature (day 6 and day 12 of gestation), plasma concentrations of cyfluthrin (day 13 of gestation) and pathology (day 13 of gestation). Statements of adherence to GLP and QA were included.

At concentrations of 0.46 µg/l and above, food intake and body-weight gain were decreased. At 2.55 µg/l and above, bloody snout, unkempt fur and piloerection were observed. At 11.9 µg/l and 12.8 µg/l (+40% oxygen) dams displayed respiratory disturbances and hypoactivity. In addition, in the group at 11.9 µg/l, dams displayed high-stepping and salivation. In the satellite groups, hypothermia and bradypnoea were found at concentrations of 0.46 µg/l and above.

No macroscopic findings were reported. At concentrations of 2.55 µg/l and above decreased placental weights (7–23%), fetal weights (11–29%) and retarded ossification of the phalanges, metacarpals, metatarsals, vertebrae, pelvis and skull, and an increased incidence of malformations were found. The nature of the malformations, with one exception (supernumerary and fused rib), were comparable to those observed in the rats in the control group in this study and in historical controls. There was no indication of a specific teratogenic potency of cyfluthrin. In the group with oxygen supplementation the embryo/fetotoxic effects were less pronounced. Therefore, the embryo/fetotoxicity of cyfluthrin after exposure by inhalation was considered to be caused by a physiological maternal compensation mechanism (hypothermia with respiratory alkalosis) after reflex bradypnoea following sensory irritation (see Pauluhn, 1992). Detectable amounts of cyfluthrin in plasma were only found in the group at the highest dose. The concentrations of cyfluthrin were low and not affected by supplementation with oxygen.

On the basis of reduced food consumption and body-weight gain, the LOAEC for maternal toxicity was 0.46 µg/l. On the basis of reduced placental and fetal weights, retardation of development and increased incidence of malformations of fetuses, the NOAEC for embryo/fetotoxicity was 0.46 µg/l air (Holzum, 1993; Schmidt, 1993).

2.6 Special studies

(a) Neurotoxicity

Hens

Cyfluthrin was tested for acute delayed neurotoxicity in White Leghorn hens in several experiments. In these experiments, symptoms of excitation and behavioural disorders, and mortality were observed in birds given single or multiple oral doses at as high as 5000 mg/kg bw. Systematic histological examinations did not reveal any evidence of treatment-related lesions to nerve tissues, nor was any effect on neuropathic target esterase (NTE) activity noted. Exposures by inhalation or by dermal application also failed to produce evidence of delayed neurotoxicity (Thyssen et al., 1981a and 1981b, 1985; Hixson, 1981; Thyssen & Kaliner 1982; Pauluhn & Kaliner, 1983; Flucke & Eben, 1985; Sacchse, 1986).

Rats

The acute oral neurotoxicity of cyfluthrin (purity, 96.1%) was assessed in an inclined plane test in groups of 5 or 10 female Wistar (Hsu Cpb: WU) rats treated by gavage. The vehicle was an aqueous Cremophor EL suspension. In the first experiment, groups of five rats received doses of 0, 0.015, 0.05, 0.1, 0.15, 0.3, 0.45, 0.5, 1, 2.5, 3, 7.5 or 9 mg/kg bw. In the second experiment, groups of 10 rats received doses of 0, 1, 2.5 or 7.5 mg/kg bw. The ability of the rats to retain their grip on a

tilted plane was assessed at several time-points between 30 min and 24 h after treatment. In addition, the rats were checked for clinical signs several times on the day of treatment. Rats from the second experiment were macroscopically examined 2 weeks after treatment. Statements of adherence to GLP and QA were included.

The slip angle was significantly reduced only in rats in the group at 9 mg/kg bw and only 2 h, but not 1 h or 6 h after administration. A dose of 7.5 mg/kg bw resulted in a marginal, statistically non-significant effect. A clinical sign of toxicity, i.e. shaking, was observed at doses of 2.5 and 3 mg/kg bw, in one and two animals respectively. The effect occurred about 2 h after administration and lasted for 1–2 h. At higher doses reduced motility, laboured breathing, increased salivation, uncoordinated gait, sternal recumbency, rolling over, narrowed palpebral fissures, digging and preening movements, diarrhoea and vocalization were also observed. The number and intensity of clinical signs increased with dose.

The NOAEL for clinical signs was l mg/kg bw. The NOAEL with regard to changes in slip angle was 7.5 mg/kg bw (Andrews, 1999).

Fifty male Sprague-Dawley rats received technical-grade cyfluthrin (purity, 95%) at a dose of 80 mg/kg bw per day as a 1.6% solution in PEG 400 by gavage on five consecutive days. Due to the onset of mild to severe symptoms of toxicity, the dose was reduced to 40 mg/kg bw per day on days 6–14. Twenty-five male rats were treated with vehicle only. The rats were observed for appearance and behaviour throughout treatment and a recovery period of 3 months. Ten treated and five control rats were killed, and their tissues examined histologically on the days 1 and 5 of treatment and 1, 2, and 3 months after cessation of treatment. At each time-point, tissues from two treated males and one control male were also examined by electron microscopy.

The treated rats showed clinical signs of intoxication (abnormal, straddled gait, slow leg movements, and excess salivation) throughout the dosing period. No symptoms were observed 5 days after cessation of treatment. Body-weight gain was reduced throughout treatment but recovered during the recovery period. Histologically, a minimal degree of axonal degeneration (swelling and demyelination) was observed in single fibres of the sciatic nerve in six out of eight rats at the first examination, in three out of eight rats 5 days to 1 month after treatment, and in two out of nine rats 2 months after treatment. A similar lesion was reported in the femoral nerve of one treated male examined on the fifth day after treatment, but was no longer seen at the end of the 3-month recovery period. No alterations were reported in any other nervous tissue or skeletal muscle examined. Electron microscopy revealed dilatation of neurotubules, accompanied by proliferation of neurofilaments and degeneration of mitochondria, which was maximal 1 month after treatment but was not seen after 3 months (Oikawa et al., 1983).

Groups of five male and five female Wistar (SPF-Cpb) rats received cyfluthrin (purity, 96.5%) at a dose of 0 or 60 mg/kg bw per day by gavage for 2 weeks. A supplementary group of male rats received doses of 0 or 50 mg/kg bw per day. Behaviour, appearance and body weights were recorded over the treatment period. At the end of treatment, rats were killed, perfused with 10% formalin and autopsied. Brain, spinal column, sciatic nerve and femoral muscle were histologically examined.

From the second day of treatment onwards, clinical signs of toxicity (tremor, altered gait, and increased vocalization) were observed in all treated animals. Four males at 60 mg/kg bw per day died between treatment days 5 and 8, but macroscopic examination revealed no specific alterations. The body-weight gain of females was not affected by treatment, but decreased by about 11% for males at 50 or 60 mg/kg bw per day. Small, fresh brain haemorrhages were seen in all four males that died. The authors concluded that the “most likely explanation is that these are the result of a terminal cardiovascular disorder with necrosis of the vascular walls”. However, since these haemorrhages

were not seen in rats in the control group, a treatment-related effect could not be ruled out (Heimann & Kaliner, 1983).

Groups of 15 male and 15 female Wistar TNO/W74 rats received cyfluthrin (purity, 83.3%) emulsified in PEG 400 by gavage daily for 35 weeks. The doses administered ranged from 30 to 80 mg/kg bw per day. The dose was varied intermittently with the purpose of inducing symptoms of acute toxicity after each dose. For the most part, doses of 60 or 80 mg/kg were given. The rats were observed daily for clinical signs. Body weights were recorded weekly. At termination clinical chemistry was performed and mixed function oxidase (MFO) activity of the liver was determined. Animals were examined macrosopically, organ weights were recorded and liver, kidney, adrenal gland, brain, spinal cord, and sciatic nerve tissues from five treated and five control rats of each sex were examined microscopically.

In the treatment group, eight males and two females died, while in the control group two males and two females died. Throughout the study treated animals displayed signs of acute toxicity (apathy, ruffled coat, and respiratory disturbances, and in some rats, increased salivation, tremor, and uncoordinated gait). No paralysis of the extremities was observed. Body-weight gain was reduced by 20–25% in males, but not in females. Clinical chemistry parameters or MFO activity were not affected by treatment. Reductions in liver and kidney weight in treated males are considered secondary to the decreased body weight. Macroscopic and histological examination revealed no treatment-related changes. However, rats that died during the treatment period were not examined (Thyssen & Vogel, 1982).

In a concisely reported study to assess the effect of treatment on neuromuscular function, groups of 10 male Wistar rats received cyfluthrin (purity, 94.5%) as a single oral dose (method of administration not specified) at 0, 0.1, 0.3 or 1.0 mg/kg bw in the first experiment and 0, 0.01, 0.03 or 0.1 mg/kg bw in the second experiment. Diazepam (5 mg/kg bw) was administered as a positive control. Cypermethrin (0.1–1.0 mg/kg bw) was used as a reference compound. The ability of the rats to retain their grip on a tilted plane was assessed at 30 min and at 2, 5 and 7 h after treatment. The experimental variable was the angle of the plane at which rats lost their grip.

Statistically significant decreases in the angle at which rats lost their grip were noted after treatment with cyfluthrin. This effect was maximal after 5 h. The effect was observed at doses of 0.03 mg/kg bw and higher. The positive control diazepam and reference chemical cypermethrin produced similar results (Polacek, 1984).

The present Meeting noted that the effects, although statistically significant (by Student t-test), were small and not strictly time- or dose-dependent. Furthermore, individual data were not presented and appropriate statistical analysis was not performed. Therefore the Meeting considered this study to be inappropriate for assessing neurotoxicity.

In a concisely reported study, the neurotoxic effects (i.e. analgesia, catalepsy, traction, orientation motility, spontaneous activity, lingomandibular reflex and neuromuscular transmission, effects on hexobarbital-induced sleep and, pentylenetetrazole-induced convulsions) of single oral doses of cyfluthrin (purity unknown) at 3, 10 and 30 mg/kg bw were assessed in groups of 5–10 male CF1 mice and male Wistar rats. The vehicle was PEG 400.

Cyfluthrin at a dose of 30 mg/kg bw followed by hexobarbital at a dose of 100 mg/kg bw (subcutaneous), induced 60% mortality in mice. No potentiation of hexobarbital-induced sleep was observed with cyfluthrin at doses of 3 and 10 mg/kg bw. Cyfluthrin at doses of 3 and 10 mg/kg bw had no muscle-relaxant, analgesic or anticonvulsive properties, and did not affect central coordination or spontaneous and orientation motility in male CF1 mice. Also in mice, a dose of 30 mg/kg bw lead to the disappearance of the righting reflex, the inability to grip the support, and prostration, but caused

no catalepsy. In rats, doses of up to and including 30 mg/kg bw caused no catalepsy and did not affect reflexes or neuromuscular transmission in male Wistar rats (Polacek, 1985).

The effects of single oral doses of cyfluthrin (purity, 94.9%) at 0, 0.1, 0.3 or 1.0 mg/kg bw (administered by gavage in Cremophor EL/water) on analgesia, traction, catalepsy, anticonvulsive properties, orientation motility, spontaneous activity and hexobarbital-induced sleep was assessed in groups of 5–10 mice and rats.

The duration and depth of hexobarbital-induced sleep in the mouse were slightly increased by cyfluthrin at 1 mg/kg bw. Lower doses had no effect. In mice, cyfluthrin exhibited no analgesic or anticonvulsant properties, and had no effect on central coordination and no protective effect against pentetrazole-induced convulsions. Cyfluthrin also had no cataleptic effect in rats and mice and did not inhibit the traction capacity of mice to any significant degree. At all doses tested (0.1–1.0 mg/kg bw), cyfluthrin induced weak, non-dose-dependent stimulation of spontaneous motility. Inhibition of the linguomandibular reflex and of neuromuscular transmission in rats was not observed (Polacek, 1982).

In a study from published literature it was reported that male Long Evans Hooded rats aged 8 weeks that had received cyfluthrin as oral doses at 0, 12.5, 25 or 50 mg/kg bw in 1 ml of corn oil 1.5 h before testing exhibited a dose-dependent reduction in spontaneous motility.

In the acoustic startling-response test, cyfluthrin (at doses of 12.5–75 mg/kg bw) reduced the amplitude of the effect and the sensitivity to background noise and increased the latent period preceding the onset of the reaction. The effects of cyfluthrin in these experiments were comparable to those of other type-II pyrethroids (Crofton & Reiter, 1988).

(b) Supplemental studies

In order to test the influence of cyfluthrin on body temperature, male Wistar rats (Bor:WISW) received single oral doses of cyfluthrin (purity, 95.3%) at 0, 125, 250 or 500 mg/kg bw by gavage. PEG 400 was used as the vehicle. Body temperature was measured at 5, 15 and 30 min and 1, 2, 2.5, 3, 3.5, 5, 5.5, 6, 6.5, 7 and 24 h after treatment. For 14 days after treatment, rats were checked daily for clinical signs. Body weight was measured at days 4, 8 and 15. At termination all animals were necropsied. Statements of adherence to GLP and QA were included.

No effect on body temperature was observed. Clinical signs (including apathy, periodic shaking, staggering gait, laboured breathing, salivation, uncoordinated movements) were observed at all doses. One animal in the group at the highest dose died (Bomann, 1991).

Studies on combination toxicity of cyfluthrin with triflumuron, methamidophos, propoxur, dichlorvos or imidacloprid did not reveal any evidence of synergism. The effects were either additive or sub-additive (Heimann, 1982b, 1983c, 1983d; Flucke, 1984a, 1984b; Krötlinger, 1994). A slight potentiation was observed on oral administration simultaneously with omethoate (Krötlinger, 1988).

In a study from published literature, a cell communication test with V79 fibroblasts, designed to indicate tumour-promoting potential, was performed. Cyfluthrin was found not to inhibit intracellular communication at non-cytotoxic concentrations (5–15 µmol/l) (Waerngard & Flodstroem, 1989).

3. Observations in humans

The effect of inhalatory exposure to cyfluthrin was assessed in 10 human subjects. In a first experiment, five men were exposed for 1 h to cyfluthrin (insecticide spray FCR 1272 0.04AE) at actual concentrations of about 2 µg/l (four subjects) or 0.09 µg/l (one subject). A second group of five

men was exposed for 1 h to actual concentrations of cyfluthrin of about 0.75 µg/l. Vital signs were recorded before, immediately after and 1.5, 2 and 3 h after the start of the exposure. In addition, in the first experiment vital signs were also recorded at 12 and 24 h after the start of the exposure, and in the second group 10 h after the start of the exposure. Electrocardiograms (ECGs) were recorded immediately before and after exposure and at 24 h (first experiment) or at 10 h (second experiment) after the start of exposure. The mucous membranes of the nose, oropharynx and eyes were examined immediately before and after exposure. For haematology and clinical chemistry, blood was sampled just before the start and 24 h (first experiment) or 10 h (second experiment) after the start of the exposure. Urine analysis was performed before and 24 h (first experiment) and 10 h (second experiment) after the start of the experiment. Statements of adherence to GLP and QA were included.

There were no clinically significant or drug-related abnormalities in vital signs, ECGs or clinical laboratory tests after either exposure session. In the first experiment, only two of the subjects tolerated exposure for the defined period of l h, mild to moderate irritation of the mucous membranes of the nose, upper respiratory tract, throat and eyes and mild hyperaemia of the nasal mucosa being observed. The effects resolved within l h after treatment.

In the second experiment, all subjects tolerated exposure for the defined period of l h. Mild irritation of the mucous membranes of the nose and throat and mild hyperaemia of the nasal mucosa were reported. The effects resolved within l h after treatment (Ruddy et al., 1998).

Cyfluthrin caused a topical skin effect, characterized by a stinging sensation in the affected areas in laboratory workers. Areas most commonly affected were the face, and mucosal tissues (e.g. prepuce). Since this chemical adheres strongly to skin and is not washed off by soap and water, it is possible to contaminate various parts of the body with cyfluthrin present on the hands (Flucke & Lorke, 1979; Miksche, 1979; Faul, 1984; Faul & Krauthausen, 1995). Yearly medical examinations of factory workers revealed no effects on body weights, haematological and urine analysis parameters, ALT and gamma glutamyl transferase (GGT) activities and thoracic organs, as examined by X-rays (Faul, 1988; Kollert, 1988; Faul & Krauthausen, 1995).

Beta-cyfluthrin

4. Toxicological studies

4.1 Acute toxicity

The results of studies of acute toxicity with beta-cyfluthrin are summarized in Table 4. The observed clinical signs (increased salivation, uncoordinated movements, increased activity and vocalization, and reduced, laboured breathing, apathy, straddle-legged gait (mostly in the rear legs), and reduced sensitivity to external stimuli) were typical of this class of pyrethroids.

Table 4. Results of studies of acute toxicity with beta-cyfluthrin

Species	Strain	Sex	Route	Formulation	LD_{50} (mg/kg bw;)	LC_{50} (µg/l)	Purity (%)	Reference
Mouse	NMRI	Male	Oral	PEG 400	91[a]	—	99.1	Heimann (1987b)[b]
		Female			165[a]			

Rat	Wistar	Male	Oral	PEG 400	380[a]	—	99.1	Heimann (1987c)[b]
		Female			651[a]			
		Male			655			
		Female			1369			
Rat	Wistar	Male	Oral	Xylene	211[a]	—	99.1	Heimann (1987d)[b]
		Female			336[a]			
		Male			307			
		Female			343			
Rat	Wistar	Male	Oral	Acetone/oil 1 : 10	84[a]	—	99.1	Heimann (1987e)[b]
		Female			77[a]			
		Male			141			
		Female			108			
Rat	??	Male	Oral	Water/Cremophor EL	16[a]	—	NS	Heimann (1984c)
		Male						
Rat	??	Male	Oral	Water/Cremophor EL	11[a]	—	NS	Heimann (1986a)
		Male						
Dog	Beagle	Male	Oral	Tylose	> 5000	—	98.5	Von Keutz (1985)
		Female			(emesis)[c]			
					> 5000			
Hen	White Leghorn	Female	Oral	Water/Cremophor EL	> 5000	—	98.5	Flucke (1985a)[b]
Rat	Wistar	Male	Dermal	PEG400	> 5000	—	98.7–99.1	Heimann (1987f)[b]
		Female			> 5000			
Rat	Wistar	Male	Dermal	Xylene	> 5000	—	98.7–99.1	Heimann (1987g)[b]
		Female			> 5000			
Rat	Wistar	Male	Inhalation (4 h)	Ethanol/PEG 400	—	90	98.5	Pauluhn (1985a)
		Female				100		
Rat	Wistar	Male	Inhalation (4 h)	Ethanol/PEG 400	—	82	97.9	Pauluhn (1988a)[b]
		Female				81		
Rat	Wistar	Male	Inhalation (4 h)	Dust	—	967	98.5	Pauluhn (1985a)
		Female				695		
Rat	Wistar	Male/ Female	Inhalation (4 h)	Dust	—	532	97.9	Pauluhn (1988a)[b]
Dog	Beagle	Male	Intravenous	Tylose	5	—	98.5	Von Keutz (1985)
		Female						
Mouse	NMRI	Male	Intraperitoneal	PEG400	18	—	98.0	Krötlinger (1988)[b]
Rat	Wistar	Male	Intraperitoneal	PEG400	917	—	99.1	Heimann (1987h)[b]
		Female						

NS, not stated.

[a] Fasted animals.

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

[c] In the study in dogs, vomiting occurred at both doses (2500 and 5000 mg/kg bw) shortly after administration.

(a) Dermal absorption

The rate and extent of dermal absorption of radioactivity was investigated after topical application of [^{14}C]beta-cyfluthrin (radiochemical purity, > 99%) at a concentration of 125 g/l or

40 g/l, in a flowable concentrate for seed treatment (FS) formulation, to male Sprague-Dawley CD rats. The animals were exposed for 8 h. Groups of five rats were killed at 8, 24, 72 and 144 h after the start of the exposure. Statements of adherence to GLP and QA were included.

During the 8-h exposure period, 0.37% and 0.48% of the applied radioactivity at the highest and lowest dose respectively were detected in excreta, carcasses (including whole blood) and untreated skin (including skin surrounding the dose site). After removal of the dose from the skin at the end of the 8-h exposure period, in the groups at the lowest and highest dose totals of 1.29% and 0.8% respectively were absorbed at 144 h (Kemp, 2004).

In a study of dermal absorption in vitro, the rate and extent of absorption was investigated after topical application of [^{14}C]beta-cyfluthrin (radiochemical purity, > 99%) at concentrations of 40 or 125 g/l to excised human and rat skin in an FS formulation. The skin samples were exposed to the test material for 8 h, after which the remaining dose was washed off the skin. Statements of adherence to GLP and QA were included.

At 24 h, the total amounts of applied radioactivity absorbed at the highest dose were 0.071% and 0.406% and at the lowest dose were 0.039% and 0.611% in human and rat skin, respectively.

The results show that rat skin is about 6 and 16 times more permeable than human skin at the highest and lowest doses used, respectively (Cage, 2004).

(b) Dermal irritation

In a study of dermal irritation, performed according to OECD guideline 404, three New Zealand White rabbits (two males, one female) received a dermal application of 0.5 g of beta-cyfluthrin (purity, 98.5%) for 4 h. Irritation was assessed according to the Draize method at 1, 24, 48, 72 and 168 h.

Very slight erythema was observed at 24 and 48 h, but not at 72 h and 168 h (Pauluhn, 1985b).

(c) Ocular irritation

In a study of eye irritation, performed according to OECD guideline 405, three male New Zealand White rabbits received 0.1 ml of beta-cyfluthrin (purity, 98.5%) in the conjunctival sac of the eye. The eyes were rinsed with saline after 24 h. Irritation was assessed according to the Draize method at 1, 24, 48, 72 and 168 h.

Slight irritation of the conjunctivae was observed at 24 and 48 h, but not at 72 and 168 h (Pauluhn, 1985b).

(d) Dermal sensitization

In a study of dermal sensitization conducted according to OECD guideline 406 and using the Magnusson & Kligman maximization test, beta-cyfluthrin (purity, 98.5%) was tested in 20 male Bor:DHWP/SPF guinea-pigs. Ten animals served as controls. In the induction phase, the animals received beta-cyfluthrin by intradermal injection (1%) on day 1 followed by a topical administration (25% w/v) after 1 week and challenge by topical administration (25%) 3 weeks after the intracutaneous injection. The vehicle was 2% Cremophor EL/saline. Statements of adherence to GLP and QA were included.

After challenge with 25% beta-cyfluthrin formulation, 10% of the animals from both the induction group and 10% of the control animals showed a slight reddening of the skin, which disappeared after 48 h (Heimann, 1986b).

In a study of dermal sensitization conducted according to OECD guideline 406 and using the Magnusson & Kligman maximization test, beta-cyfluthrin (purity, 98.6%) was tested in 20 male Hsd/Win:DH guinea-pigs. Ten animals served as controls. In the induction phase, the animals received beta-cyfluthrin by intradermal injection (5%) on day 1 followed by a topical administration (50% w/v) after 1 week and challenge by topical administration (25 or 50%) 3 weeks after the intracutaneous injection. The vehicle was 2% Cremophor EL/saline. Statements of adherence to GLP and QA were included.

No dermal reactions were observed in either the induction or the control group (Vohr, 1994b).

4.2 Short-term studies of toxicity

(a) Oral administration

Rats

In a 4-week study of toxicity performed according to OECD guideline 407, groups of 15 male and 15 female Wistar rats were treated orally with beta-cyfluthrin (purity, 98.6%) at a dose of 0, 0.25, 1, 4 or 16 mg/kg bw per day by gavage. Additional groups of 15 males and 15 females were treated for 4 weeks and then kept untreated for a 4-week recovery period. Rats were observed daily for clinical signs and mortality and body weights were recorded weekly. Haematology, clinical chemistry, urine analysis and measurements of enzyme levels (*N*-demethylase, *O*-demethylase, cytochrome P450) in the liver were conducted on five males an five females per dose at the end of the 28-day treatment interval, and at the end of the 4-week recovery period. At termination, 10 males and 10 females per dose were macroscopically examined and organs were weighed. Histology was performed on organs and tissues, including nervous tissue, of five males and five females per dose. Statements of adherence to GLP and QA were included.

At the highest dose, 11 males and 12 females died. At 4 mg/kg bw per day, rats displayed increased motility and grooming and digging movements throughout the treatment period, starting on the first day of treatment. These symptoms occurred 30–80 min after application and lasted for about 4 h. In this group, salivation was also observed. At 16 mg/kg bw per day, ataxia, straddled-gait, dyspnoea, apathy and dacryohemorrhoea were also observed. No effects of treatment on body weight, clinical chemistry, haematology, urine analysis and liver enzymes were observed. Relative liver, adrenal and testis weights were increased in males of the group at the highest dose. In females of the group at the highest dose, relative lung weight was increased. Macroscopic and histopathological examinations and special histopathological studies of nervous tissues did not indicate treatment-related effects. At the end of the 4-week recovery period, no effects were observed.

On the basis of the clinical signs, the NOAEL was 1 mg/kg bw per day (Heimann & Majeed, 1988).

Groups of 15 males and 15 female Wistar rats received diets containing beta-cyfluthrin (purity, 99.7%) at a concentration of 0, 30, 125, or 500 ppm (equal to 0, 2.3, 9.5 and 38.9 mg/kg bw per day in males and 0, 2.5, 10.9 and 42.9 mg/kg bw per day in females) for 13 weeks. In addition, groups of 15 males anf 15 females were fed diets containing beta-cyfluthrin at a concentration of 0 or 500 ppm (equal to 0 and 37.0 mg/kg bw per day in males and 0 and 43.0 mg/kg bw per day in females) for 13 weeks and were then kept on control diet for an additional 4 weeks. Rats were examined daily for mortality and clinical signs. Body weights and food and water consumption were recorded weekly. Clinical chemistry, haematology and urine analysis were conducted after 1 and 3 months and at the end of the recovery period. After 13 weeks and at the end of the recovery period, a tooth and femur

were removed from five males and five females per dose for determination of fluoride content. In addition, the activity of microsomal enzymes in liver was determined in these rats. Ophthalmoscopy was performed at weeks 1 and 11 during treatment, and at week 15 in rats in the recovery groups. At the end of the treatment or recovery period, the rats were killed, macroscopically and histologically (including bone-marrow smears) examined and organs were weighed. Statements of adherence to GLP and QA were included.

No effect of treatment on mortality, clinical signs, food and water consumption and body-weight gain were observed at doses of up to and including 125 ppm. At 500 ppm, rats exhibited an uncoordinated gait and impaired general condition. Food (± 12%) and water consumption (± 18%) and body weight (± 8%) were reduced during the first week of treatment. No treatment-related effects were observed on any of the other parameters tested.

On the basis of the clinical signs and reductions in food and water consumption and body-weight gain of rats during the first week of treatment, the NOAEL was 125 ppm, equal to 9.5 mg/kg bw per day (Suberg & Wood, 1988).

Dogs

In a 13-week dietary study, groups of four male and four female beagle dogs received beta-cyfluthrin (purity, 99.2%) at a concentration of 0, 10, 60 or 360 ppm (equivalent to 0, 0.3, 1.3 and 9 mg/kg bw per day). Clinical signs and food consumption were recorded daily. Body weights were assessed weekly. Measurements of reflexes, body temperature and pulse rate, haematology, clinical chemistry and urine analysis were performed before testing and during weeks 4, 7 and 13 of treatment. Ophthalmoscopy was performed before testing and during weeks 7 and 13. At the end of the treatment or recovery period, the dogs were killed, macroscopically and histologically examined (including bone-marrow smears) and organs were weighed. In addition the activity of microsomal enzymes in liver and the fluoride content in bones and teeth were determined. Statements of adherence to GLP and QA were included.

The treatment induced no mortalities. Animals of the group at the highest dose occasionally displayed unsteady, staggering gait, intermittent buckling of the hind legs, vomiting and diarrhoea for short periods after feeding. Body weight was slightly decreased (7%) in females of the group at the highest dose. No other treatment-related effects were observed.

On the basis of clinical signs in both sexes, the NOAEL was 60 ppm, equivalent to 1.3 mg/kg bw per day (Von Keutz, 1987).

(b) Exposure by inhalation

Rats

In a dose range-finding study, groups of 10 male and 10 female Wistar rats were exposed to aerosols containing beta-cyfluthrin (purity, 98%) at actual concentrations of 0, 0.3, 3.8 or 28.0 µg/l air by nose-only inhalation for 6 h per day for five consecutive days, followed by a 2-week recovery period. The rats were observed daily for clinical signs, but not during exposure. Body weights were assessed before testing, on days 5 and 8 and once per week thereafter. At the end of the recovery period, the rats were necropsied. Statements of adherence to GLP and QA were included.

At a concentration of 3.8 µg/l air, transient clinical signs (unkempt fur and piloerection) were noted. Rats in the group at the highest dose still exhibited these signs in the morning before they were again exposed. During the exposure period, body-weight gain in the rats at the highest dose was reduced (7%). On the basis of the clinical signs, the NOAEC was 0.3 µg/l air (Pauluhn, 1988b).

Groups of 10 male and 10 female Wistar rats were exposed to aerosols containing beta-cyfluthrin (purity, 97.9%) at actual concentrations of 0, 0.2, 2.7 or 23.5 µg/l air by nose-only

inhalation for 6 h per day for five consecutive days. The rats were observed daily for clinical signs, but not during exposure. Body weights were assessed weekly. Ophthalmoscopy was performed before testing and towards the end of the study. In the last third of the study, five male rats of each group were subjected to lung function tests. Clinical chemistry, haematology and urine analysis were performed at the end of the study. At termination, the rats were necropsied, selected organs were weighed and a wide range of organs were examined histologically. At termination liver tissue was also sampled for determination of cytochrome P450, *N*-demethylase and *O*-demethylase activity and triglyceride concentrations. Statements of adherence to GLP and QA were included.

At the highest concentration, animals displayed slight to moderate clinical signs (unkempt fur, piloerection, hyperactivity or, partially, hypoactivity). At concentrations of 2.7 µg/l air and higher, body-weight gains were reduced in both sexes (7–11%). At 23.5 µg/l, air the rats had reduced absolute and relative thymus and spleen weights, and reduced leukocyte and lymphocyte counts. No other toxicologically relevant effects were observed.

On the basis of reduced body-weight gains in both sexes, the NOAEC was 0.2 µg/l air (Pauluhn, 1989c).

4.3 Genotoxicity

The results of tests for genotoxicity with beta-cyfluthrin are summarized in Table 5. The Meeting concluded that beta-cyfluthrin is unlikely to be genotoxic.

Table 5. Results of studies of genotoxicity with beta-cyfluthrin

End-point	Test object	Concentration[a]	Cyfluthrin content (%)	Results	Reference[b]
In vitro					
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535 and TA1537	20–12 500 µg/plate (± S9)	98.5	Negative	Herbold (1986)
Gene mutation	CHO K1-BH4 cells, *Hprt* locus	20–100 µg/ml (± S9)	99.6	Negative	Lehn (1988)
Chromosomal aberration	Human lymphocytes	500–5 000 µl/ml (± S9)	98.8	Negative	Herbold (1988b)
Unscheduled DNA synthesis	Rat primary hepatocytes	1.01–1 010 µg/ml	99.5	Negative	Cifone (1987)
In vivo					
Micronucleus formation	Mouse bone marrow	80 mg/kg bw, oral	99.6	Negative	Herbold (1988c)

[a] Positive and negative (solvent) controls were included in all studies.
[b] Statements of adherence to GLP and QA were included in all studies.

4.4 Reproductive toxicity: developmental toxicity

Rats

In a study of developmental toxicity, inseminated Wistar rats were given technical-grade beta-cyfluthrin (purity, 96.5%–97.3%) at a dose of 0, 2.7, 9.4, and 42.0 mg/kg bw per day by oral gavage on days 6 to 15 of gestation. The animals were observed daily for mortality and clinical signs. Body

weights and food consumption were recorded on days 0 (body weight only), 2, 4, 6–16, 18 and 20 of gestation. On day 20 of gestation, dams were killed and necropsied and ovaries and gravid uteri were examined. About one half the live fetuses in each litter were examined for visceral changes, the other half for skeletal effects. Statements of adherence to GLP and QA were included.

Severe maternal toxicity, including mortality, was observed in the group at the highest dose. Clinical signs included hypoactivity, locomotor incoordination, and salivation. In the group at the highest dose, body-weight gain (−55%) and food consumption were decreased. The statistically non-significant reduction in body-weight gain in females at the intermediate dose (−9%) was considered to be not biologically significant. In the group at the highest dose, a statistically significant decrease (9%) in fetal weight was observed. In these fetuses from the group at the highest dose, incompletely ossified frontal bones, sacral arches, metacarpals, and second sternebrae, unossified caudal arches, fifth sternebrae, and xiphoid and enlarged anterior fontanelle were also found, which were considered to be secondary to maternal toxicity at this dose. No other treatment-related effects on reproductive or developmental end-points, including pre/postimplantation loss, resorptions and visceral development were observed.

On the basis of increased mortality, clinical signs, decreased body-weight gain and food consumption, the NOAEL for maternal toxicity was 9.4 mg/kg bw per day. On the basis of the reduced fetal weight and the retarded ossification of the bones, the NOAEL for embryo/fetotoxicity was 9.4 mg/kg bw per day (Astroff, 1996).

4.5 Special studies: neurotoxicity

Rats

Groups of 12 male and 12 female fasted Fischer 344 rats were given technical-grade beta-cyfluthrin (purity, 96.9–97.3%) as a single dose at 0, 0.5, 2 or 10 mg/kg bw by gavage. The vehicle was 1% Cremophor EL in deionized water. The rats were observed daily for clinical signs and mortality. Body weight was measured weekly. The animals were tested in a functional observational battery (FOB) and motor activity test 1 week before testing and 2 h and 7 days and 14 days after treatment. At termination on day 15, all rats were necropsied and brains were weighed. Six males and six females per dose were selected for perfusion and tissue collection. Skeletal muscle, peripheral nerves, eyes (with optic nerves) and tissues from the central nervous system of the rats in the control group and at the highest dose were examined histologically. Statements of adherence to GLP and QA were included.

No deaths occurred. Beta-cyfluthrin at a dose of 2 mg/kg bw, induced perianal staining during the first 5 days after treatment. At 10 mg/kg bw, perianal, oral and urine stains were observed. Body weight was not affected by treatment. In the FOB, 2 h after treatment, rats at the intermediate dose displayed a decreased approach response (both sexes), oral stains (males only) and a decreased activity (females only). In the group at the highest dose, rats displayed uncoordinated gait, decreased activity, flattened posture, repetitive pawing motion, decreased approach and touch responses, impaired righting, salivation and oral, urine and perianal stains. In addition, in males a decreased body temperature, writhing behaviour, decreased tail pinch response and prolapsed penis was observed while females displayed slight muscle fasciculations. Motor and locomotor activities were decreased on day 0 in males at 10 mg/kg bw and in females at 2 and 10 mg/kg bw. All signs of toxicity had resolved on day 7. No effects of treatment on brain weight, macroscopic and histological parameters were observed.

On the basis of the perianal staining and the effects in the FOB and motor activity tests, the NOAEL for beta-cyfluthrin was 0.5 mg/kg bw (Sheets et al., 1997).

Groups of 12 male and 12 female Fischer 344 rats were given diets containing beta-cyfluthrin (purity, 96.5–97.3%) at a concentration of 0, 30, 125 or 400 ppm (equal to 0, 2.0, 8.9 and 26.8 mg/kg bw per day for males and 0, 2.3, 9.4 and 30.8 mg/kg bw per day for females) for 13 weeks. The rats were checked daily for clinical signs and mortality. A detailed clinical examination was performed once per week. Body weight and food consumption were determined weekly. The animals were tested in a FOB and a test for motor activity 1 week before and 4, 8 and 13 weeks after the start of treatment. Ophthalmoscopy was performed before treatment and in week 12. After 13 weeks of treatment, all rats were killed and examined macroscopically. Six male and six females per group were selected for perfusion and tissue collection. From these rats, the brain, spinal cord, eyes, sciatic, tibial and sural nerves, gasserian ganglion, and gastrocnemius muscle were collected for histological examination. The brains of these animals were weighed. These tissues from rats in the control group and at the highest dose were examined histologically. Statements of adherence to GLP and QA were included.

No mortality occurred. Beta-cyfluthrin induced clinical signs throughout the treatment period in males at 125 ppm and in both sexes at 400 ppm. In males of the group at 125 ppm, self-induced lesions from scratching were observed. These were considered to be the consequence of paresthesias after absorption of beta-cyfluthrin through the skin. At the highest dose, clinical signs consisted of ataxia and repetitive chewing movements in males and ataxia, repetitive pawing, increased reactivity, increased activity and red nasal stains in females. Body-weight gain was reduced in males of the group at 400 ppm (−31%) and in females of the groups at 125 ppm (−21%) and 400 ppm (−39%). In these groups, food consumption was also decreased. At 400 ppm, FOB testing revealed repetitive chewing, uncoordinated gait, decreased fore- and hindlimb grip strength in both sexes, and additionally increased reactivity, a slightly exaggerated auditory response, a slight decrease in body temperature and an uncoordinated righting response in females. Motor and locomotor activities were not affected by treatment at any dietary concentration. There were no treatment-related ophthalmic findings. No effects of beta-cyfluthrin on brain weight and macroscopic or histological parameters were found.

On the basis of the decreased body-weight gain and food consumption in females of the group at 125 ppm, the NOAEL was 30 ppm, equal to 2.3 mg/kg bw per day (Sheets & Hamilton, 1997).

In a study of developmental neurotoxicity, groups of 30 mated female Wistar rats were given diets containing technical-grade beta-cyfluthrin (purity, 95.1–97.6%) at a concentration of 0, 29, 133 or 215 ppm (equal to 0, 2.4, 11.0 and 17.8 mg/kg bw per day during gestation and 0, 5.9, 25.4 and 40.9 mg/kg bw per day during lactation) from day 0 of gestation until day 21 of lactation. The dams were subjected daily to a detailed examination for clinical signs. All rats were tested in a detailed observational battery (in the home cage, during handling and in an open field) on day 6 and day 21 of gestation, while the same test was performed on 10 rats per group on day 11 and day 21 of lactation. Body weight and food consumption were assessed on days 0, 6, 13 and 20 of gestation and days 0, 4 (body weight only), 7, 14 and 21 of lactation. At postnatal day 4, litters were culled to eight pups. The pups were examined daily for clinical signs and selected pups (about 16 males and 16 females per group) were subjected to a detailed observational battery on postnatal days 4, 11, 21, 35, 45 and 60. Body temperature was assessed in the dams and selected pups on postnatal days 10, 15, 18 and 21. In the pups, body weight (from postnatal day 0) and food consumption (from postnatal day 28) were assessed weekly. In addition preputial separation or vaginal patency, motor activity (postnatal days 13, 17, 21 and 60), acoustic startle habituation (postnatal days 22, 38 and 60) learning and memory (postnatal days 22, 29, 60) were assessed. Ophthalmoscopy was performed in pups at age 7–8 weeks. Neural tissues were collected on postnatal day 21 and at study termination for microscopic examination and morphometry. Statements of adherence to GLP and QA were included.

In females at the highest dose, body-weight gain was reduced during the first week of gestation (−56%) and during the first 2 weeks of lactation (−18%). In this group food consumption was reduced (9–11%) during the second and third week of lactation. No other treatment-related effects were observed.

In the offspring, a reduced body-weight gain was found in males at the highest dose and females from postnatal days 4 to 11. Acoustic startle habituation was reduced in males at the highest dose at postnatal day 22. No treatment-related effects on the other parameters were observed.

On the basis of the reduced body-weight gain and food consumption in the dams, the NOAEL for maternal toxicity was 133 ppm, equal to 11.0 mg/kg bw per day. On the basis of the reduced body weight during lactation and the reduced startle habituation at postnatal day 22, observed in the pups at the highest dose, the NOAEL for offspring toxicity was 133 ppm. Although the reduced startle habituation in the pups was observed during or just after the lactation period, when the compound intake of the dams was higher, it could be excluded that the observed effects were the result of exposure during gestation. Therefore, the NOAEL of 133 ppm for offspring toxicity was considered to be equal to 11.0 mg/kg bw per day (Sheets & Lake, 2003).

5. Studies on metabolites

The results of studies of acute toxicity and genotoxicity are summarized in Table 6. The LD_{50} studies were performed in fasted rats and in non-fasted mice.

Table 6. Results of studies of acute toxicity and genotoxicity with metabolites of cyfluthrin

Metabolite		LD_{50} (mg/kg bw)		Result of *S.typhimurium* test (reverse mutation)[f]	References
	Species	Males	Females		
3-Phenoxy-4-fluoro-benzyl alcohol	Rat	1589	1600–1800	Negative	Krötlinger (1987a); Herbold (1987a)
3-Phenoxy-4-fluoro-benzaldehyde	Rat	1248	1040	Negative	Thyssen (1981); Herbold (1985)
3-Phenoxy-4-fluorobenzoic acid	Rat	> 5000	> 5000	—	Krötlinger (1986a)
3(4′-Hydroxyphenoxy)-4-fluorobenzoic acid	Rat	> 1000	> 1000	Negative	Krötlinger (1987b); Herbold (1987b)
3-Phenoxy-4-fluorobenzoic acid amide	Rat	> 5000	> 5000	Negative	Krötlinger (1986b); Herbold (1988e)
Metabolite 1[a]	Rat	> 2500	> 2500	Negative	Krötlinger (1986c); Herbold (1988d)
Metabolite 2[b]	Rat	> 2500	> 2500	—	Krötlinger (1986d)
Metabolite 3[c]	Mouse	370[e]	—	—	Gaughan et al. (1977)
Metabolite 4[d]	Mouse	210[e]	—	—	Gaughan et al. (1977)

[a] Metabolite 1: +,-(*R,S*)-α-Carboxy-[3-phenoxy-4-fluoro]benzyl-1-(*R,S*)-*trans*-3-(2′,2′-dichloroethen-1′-y1)-2,2-dimethylcyclo-propanecarboxylic acid ester

[b] Metabolite 2: +,-(*R,S*)-α-Carboxamido-[3-phenoxy-4-fluoro]benzyl-1-(*R,S*)-*trans*-3-(2,2-dichloroethen-1-y1)-2,2-dimethyl-cyclopropanecarboxylic acid ester

[c] Metabolite 3: *cis*-3-(2′,2′-Dichloroethen-1′-yl)-2,2-dimethyl-cyclopropanecarboxylic acid

[d] Metabolite 4: *trans*-3-(2′,2′-Dichloroethen-1′-y1)-2,2-dimethyl-cyclopropanecarboxylic acid

[e] Administered intraperitoneally administration in 20–50 µl of methoxytriglycol to Swiss mice.

[f] Salmonella/microsome test for mutagenic activity.

The metabolites of cyfluthrin studied (both confirmed and hypothetical) exhibit weak to moderate toxicity after oral administration to the rat and after intraperitoneal administration to the mouse. The data indicate that their acute toxicity is lower than that of the parent compound (see Table 2). No evidence of mutagenic potential was found for any of the metabolites studied.

Comments

Biochemical aspects

In rats, cyfluthrin is rapidly absorbed and distributed. Peak concentrations in the blood were reached after 1 or 6 h, depending on the vehicle used. About 98% of the radiolabel was eliminated in the urine and faeces within 48 h after oral administration, with an initial half-life of about 3 h. Similar amounts were eliminated after intravenous administration. The ratio of excretion in urine : faeces was higher in males (3 : 1) than in females (3 : 2). About one third of the administered dose was excreted in bile. The highest concentrations of radiolabel were found in fat, ovaries, adrenal, liver and spleen. Repeat-dosing experiments yielded similar results. In rats, the major metabolic transformation is ester hydrolysis to a 3-phenoxy-4-fluorobenzyl alcohol intermediate and a permethric acid moiety. After ester hydrolysis, the benzyl alcohol moiety is oxidized to the free 3-phenoxy-4-fluorobenzoic acid metabolite, which can either be conjugated with glycine or oxidized to give 4'-hydroxy-3-phenoxy-4-fluorobenzoic acid.

Toxicological data

The acute oral LD_{50} values of cyfluthrin and beta-cyfluthrin in rats ranged from 11 to > 1000 mg/kg bw, depending on the vehicle used and the feeding status of the animals. The observed clinical signs (increased salivation, uncoordinated movements, increased activity and vocalization, and reduced, laboured breathing, apathy, straddle-legged gait (mostly in the rear legs), and reduced sensitivity to external stimuli) are typical of this class of pyrethroids. In rats, the inhalation LC_{50} values ranged from 0.047 to > 1 mg/l, and the dermal LD_{50} values were > 5000 mg/kg bw. Cyfluthrin is not irritating to the skin, is slightly irritating to the eyes and is not a skin sensitizer.

The critical end-points induced by cyfluthrin and beta-cyfluthrin are neurotoxicity and reduction in body weight.

Several short-term studies of oral toxicity with cyfluthrin were available for mice, rats and dogs. In a 4-week study in mice, the NOAEL was 300 ppm (equal to 43.1 mg/kg bw per day) on the basis of histological changes in the liver and the submaxillary gland. In one 4-week dietary study and two 3-month dietary studies, the overall NOAEL was 100 ppm (equal to 8.3 mg/kg bw per day) on the basis of a reduction in blood glucose concentration. In a 4-week gavage study, the NOAEL was 20 mg/kg bw per day on the basis of mortality, reduced body weight, increased liver and adrenal weight and clinical signs. In one 6-month study and two 12-month studies in dogs, the overall NOAEL was 200 ppm (equal to 6.5 mg/kg bw per day) on the basis of clinical signs and reductions in body-weight gain.

The effects of short-term oral exposure to beta-cyfluthrin were assessed in rats and dogs. In a 4-week gavage study in rats, the NOAEL was 1 mg/kg bw per day on the basis of clinical signs (increased motility, grooming and digging movements) at 4 mg/kg bw per day, which had already been observed on the first day of administration. In a 13-week dietary study in rats, the NOAEL was 125 ppm (equal to 9.5 mg/kg bw per day) on the basis of clinical signs, and reductions in body-weight gain, food and water consumption. In a 13-week dietary study in dogs, the NOAEL was 60 ppm (equal to 1.3 mg/kg bw per day) on the basis of clinical signs.

The dermal toxicity of cyfluthrin was assessed in 3-week studies in rats and rabbits. In rats, the NOAEL of 340 mg/kg bw per day was identified on the basis of a reduction in food consumption and clinical signs. In rabbits, no toxicologically relevant effects were observed at doses of up to and including 250 mg/kg bw per day, i.e. the highest dose tested.

In short-term studies of toxicity after inhalation of cyfluthrin and beta-cyfluthrin in rats, with a duration ranging from 5 days to 13 weeks, the overall NOAEC of 0.09 µg/l (equivalent to an inhalational dose of about 0.02 mg/kg bw per day[2]) was identified on the basis of reduced body-weight gain and behavioural changes. The high toxicity of cyfluthrin administered by inhalation, compared with oral exposure, was considered to be caused by the local effects of cyfluthrin on the respiratory system.

In long-term studies of toxicity and carcinogenicity, mice and rats were treated with cyfluthrin at dietary concentrations of up to 1400 and 450 ppm respectively. Statistically significant reductions in body-weight gain (> 10%), which were not related to reductions in food consumption, were consistently found in all studies. In two studies in mice, an overall NOAEL of 200 ppm, equal to 38.4 mg/kg bw per day was identified on the basis of reductions in body-weight gain in females, and macroscopic (crusty zones of the skin of the ear) and histological changes (acanthosis, chronic active inflammation, ulcer, debris of the skin of the ear) in males. The macroscopic and histological effects in the ear are likely to be due to the scratching of the skin by the animals in reaction to local paraesthesia, which is a characteristic of this class of compounds. In two studies in rats, the overall NOAEL was 150 ppm (equal to 6.2 mg/kg bw per day) on the basis of decreases in body-weight gain. The small reductions in body-weight gain (5–6%) observed at lower doses in both studies were not considered to be biologically significant. No evidence for a tumorigenic effect of cyfluthrin was found.

The Meeting concluded that cyfluthrin is not carcinogenic in rodents.

Cyfluthrin and beta-cyfluthrin gave negative results in an adequate range of tests for genotoxicity in vitro and in vivo. The Meeting concluded that cyfluthrin and beta-cyfluthrin are unlikely to be genotoxic.

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

In three multigeneration dietary studies with cyfluthrin in rats, the overall NOAEL for parental toxicity was 125 ppm (equal to 9 mg/kg bw per day) on the basis of reductions in body-weight gain at 400 ppm (equal to 29 mg/kg bw per day), and a borderline reduction in body-weight gain at 150 ppm (equal to 11.4 mg/kg bw per day). The overall NOAEL for offspring toxicity was 50 ppm (equal to 7 mg/kg bw per day) on the basis of coarse tremors in pups during lactation. The overall NOAEL for reproductive effects was 450 ppm, equal to 34.7 mg/kg bw per day, the highest dose tested.

The effect of oral exposure to cyfluthrin on prenatal development was investigated in rats and rabbits. In two studies of developmental toxicity in rats treated by gavage, the overall NOAEL for maternal toxicity was 3 mg/kg bw per day on the basis of clinical signs. The overall NOAEL for fetal toxicity was 30 mg/kg bw per day, i.e. the highest dose tested. In a study of developmental toxicity in rabbits treated by gavage, the NOAEL for maternal and embryo/fetotoxicity was 15 mg/kg bw per day, on the basis of two abortions and one case of complete resorption in the group of 15 dams treated with cyfluthrin at a dose of 45 mg/kg bw per day. In a second study in rabbits, the NOAEL for maternal toxicity was 20 mg/kg bw per day on the basis of reduced food consumption and body-weight gain. The NOAEL for fetotoxicity was 20 mg/kg bw per day on the basis of increased post-implantation loss.

[2] Exposure was 6 h per day. Twenty-four hour respiratory volume for rats is 0.96 ml/kg bw (Zielhuis & Van der Kreek, 1979).

The increased incidence of malformations observed in a study of developmental toxicity in rats treated with cyfluthrin by inhalation was considered to be secondary to effects on pulmonary function.

In a study of developmental toxicity in rats treated with beta-cyfluthrin by gavage, the NOAEL for maternal toxicity was 9.4 mg/kg bw per day on the basis of a reduction in body-weight gain, clinical signs and mortality. The NOAEL for embryo/fetotoxicity was 9.4 mg/kg bw per day on the basis of reduced fetal weight and retarded ossification.

Cyfluthrin did not cause delayed neurotoxicity in hens. In a study of acute neurotoxicity in rats treated by gavage, the NOAEL was 1 mg/kg bw per day on the basis of mild clinical signs of toxicity (shaking) observed in one and two animals at 2.5 and 3 mg/kg bw, respectively. A range of additional studies of neurotoxicity with repeated high doses of cyfluthrin (30–80 mg/kg bw per day) administered by gavage also demonstrated clinical signs of (neuro-) toxicity, including disturbed gait, salivation, tremor and apathy.

Occasionally, in some of the short-term studies of toxicity and studies of neurotoxicity in rats, marked acute toxicity induced by high doses of cyfluthrin was accompanied by limited swelling and fragmentation of myelin, which was reversed within 1–3 months after cessation of treatment. The Meeting concluded that cyfluthrin does not cause irreversible neurological damage.

The (developmental) neurotoxicity of beta-cyfluthrin was investigated in rats. In a study in which single doses were administered by gavage, the NOAEL was 0.5 mg/kg bw per day on the basis of perianal staining, effects in the functional observational battery (decreased approach response in both sexes, oral staining in males and a decreased activity in females) and decreased motor and locomotor activities in females. In a 13-week dietary study, the NOAEL was 30 ppm (equal to 2.3 mg/kg bw per day) on the basis of reductions in body-weight gain and food consumption in females.

In a study of developmental neurotoxicity in rats, the NOAEL for maternal toxicity was 133 ppm (equal to 11 mg/kg bw per day) on the basis of reduced body weight and food consumption. The NOAEL for offspring toxicity was 133 ppm (equal to 11 mg/kg bw per day) on the basis of reduced body-weight gain during lactation and reduced startle habituation at postnatal day 22.

A number of pharmacological studies with cyfluthrin were considered not to be useful for the purpose of dietary risk assessment.

A limited number of studies of acute toxicity and genotoxicity with some metabolites[3] of cyfluthrin were performed. The acute toxicity of the metabolites studied was lower than that of the parent compound. No evidence of mutagenic potential was found for any of the metabolites investigated.

Cyfluthrin caused a topical skin effect, characterized by a stinging sensation in the affected areas in laboratory workers. The areas most commonly affected were the face and mucosal tissues. Annual medical examinations of factory workers revealed no effects on body weights, haematological and urine analysis parameters, ALT and GGT activities and thoracic organs, as examined by X-rays.

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[3] Metabolite 1: +,-(*R,S*)-α-Carboxy-[3-phenoxy-4-fluoro]benzyl-1-(*R,S*)-*trans*-3-(2′,2′-dichloroethen-1′-yl)-2,2-dimethylcyclo-propanecarboxylic acid ester
Metabolite 2: +,-(*R,S*)-α-Carboxamido-[3-phenoxy-4-fluoro]benzyl-1-(*R,S*)-*trans*-3-(2,2-dichloroethen-1-yl)-2,2-dimethyl-cyclopropanecarboxylic acid ester
Metabolite 3: *cis*-3-(2′,2′-Dichloroethen-1′-yl)-2,2-dimethyl-cyclopropanecarboxylic acid
Metabolite 4: *trans*-3-(2′,2′-Dichloroethen-1′-yl)-2,2-dimethyl-cyclopropanecarboxylic acid

The Meeting concluded that the present database was adequate to characterize the potential hazard of cyfluthrin and beta-cyfluthrin to fetuses, infants and children.

Evaluation of the available data showed that the toxicological profiles of cyfluthrin and beta-cyfluthrin appeared to be qualitatively similar. With respect to neurotoxicity, beta-cyfluthrin, being the biologically active component of cyfluthrin, was more potent than cyfluthrin. The Meeting concluded that the database for cyfluthrin was adequate to apply to beta-cyfluthrin. Therefore no additional studies on beta-cyfluthrin were necessary.

Toxicological evaluation

The Meeting considered establishing a group ADI of 0–0.06 mg/kg bw on the basis of an overall NOAEL of 6.2 mg/kg bw per day for reductions in body-weight gain observed in a 2-year dietary study with cyfluthrin in rats, and using an safety factor of 100. The overall LOAEL for this effect was 9.4 mg/kg bw per day, observed in a 13-week dietary study with beta-cyfluthrin in rats. Since the mode of action for reduction in body-weight gain was unknown, the Meeting considered it appropriate to apply the default 100-fold safety factor for this end-point. This was considered to be adequately protective against the neurotoxic effects of beta-cyfluthrin in dietary studies.

The Meeting established a group ARfD for cyfluthrin and beta-cyfluthrin of 0.04 mg/kg bw based on a NOAEL of 1 mg/kg bw for findings of acute neurotoxicity observed in a 4-week study with beta-cyfluthrin administered by gavage, and using a safety factor of 25. This overall NOAEL was supported by the NOAEL of 0.5 mg/kg bw identified on the basis of slight neurotoxic effects at 2 mg/kg bw in a study of neurotoxicity in rats given single doses of beta-cyfluthrin by gavage, and the NOAEL of 1 mg/kg bw identified on the basis of slight clinical effects at 2.5 mg/kg bw in a study in rats given single doses of cyfluthrin by gavage. Furthermore, this overall NOAEL is in line with the threshold dose of 0.9 mg/kg bw for acute effects of beta-cyfluthrin on motor activity (Wolansky et al., 2006). Since the neurotoxicity induced by (beta-) cyfluthrin is dependent on the C_{max} and is reversible, the Meeting considered it appropriate to apply a chemical-specific adjustment factor of 25 for this end-point.

The Meeting noted that the proposed ADI based on body-weight effects in studies with repeated doses was higher than the ARfD. Therefore, the ADI was established at 0–0.04 mg/kg bw, i.e. the same value as the ARfD.

Levels relevant for risk assessment

(a) Cyfluthrin

Species	Study	Effect	NOAEL	LOAEL
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	150 ppm, equal to 6.2 mg/kg bw per day	450 ppm, equal to 19.2 mg/kg bw per day—[c]
		Carcinogenicity	450 ppm, equal to 19.2 mg/kg bw per day[c]	
	Two-generation study of reproductive toxicity[a]	Parental	50 ppm, equal to 3.8 mg/kg bw per day	150 ppm, equal to 11.4 mg/kg bw per day[d]

		Offspring toxicity	50 ppm, equal to 5.1 mg/kg bw per day	150 ppm, equal to 14.0 mg/kg bw per day
		Reproductive toxicity	450 ppm, equal to 34.7 mg/kg bw per day[c]	—[c]
	Two-generation study of reproductive toxicity[a]	Parental	125 ppm, equal to 9 mg/kg bw per day	400 ppm, equal to 29 mg/kg bw per day
		Offspring toxicity	50 ppm, equal to 7 mg/kg bw per day	150 ppm, equal to 19 mg/kg bw per day
		Reproductive toxicity	400 ppm, equal to 29 mg/kg bw per day[c]	—[c]
	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	—[c]
		Fetotoxicity	10 mg/kg bw per day	—[c]
	Acute neurotoxicity[b]	Neurotoxicity	1 mg/kg bw	2.5 mg/kg bw
Dog	6-month study of toxicity[a]	Toxicity	200 ppm, equal to 6.5 mg/kg bw per day	600 ppm, equal to 19.9 mg/kg bw per day

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.
[d] Actual NOAEL is likely higher given that the effects were borderline at the LOAEL.

(b) Beta-cyfluthrin

Species	Study	Effect	NOAEL	LOAEL
Rat	4-week study of toxicity[b]	Toxicity	1 mg/kg bw per day	4 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	9.4 mg/kg bw per day	42 mg/kg bw per day
		Fetotoxicity	9.4 mg/kg bw per day	42 mg/kg bw per day
	Acute neurotoxicity[b]	Neurotoxicity	0.5 mg/kg bw	2 mg/kg bw
	13-week study of neurotoxicity[a]	Neurotoxicity	30 ppm, equal to 2.3 mg/kg bw per day	125 ppm, equal to 9.4 mg/kg bw per day
	Developmental neurotoxicity[a]	Maternal toxicity	133 ppm, equal to 11 mg/kg bw per day	215 ppm, equal to 17.8 mg/kg bw per day
		Fetotoxicity	133 ppm, equal to 11 mg/kg bw per day	215 ppm, equal to 17.8 mg/kg bw per day
Dog	3-month study of toxicity[a]	Toxicity	60 ppm, equal to 1.3 mg/kg bw per day	360 ppm, equal to 9 mg/kg bw per day

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

Estimate of acceptable daily intake for humans

0–0.04 mg/kg bw

Estimate of acute reference dose

0.04 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 cyfluthrin or beta-cyfluthrin

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of absorption	Rapid and extensive (rats)
Distribution	Highest concentrations in liver, kidney, adrenals, spleen (rats)
Potential for accumulation	Low
Rate and extent of excretion	Rapid (75–91% in urine within 24 h in rats)
Metabolism in animals	Major metabolites: 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCVA), 3-phenoxy-4-fluorobenzoic acid, 4´-hydroxy-3-phenoxy-4-fluorobenzoic acid (rats)
Toxicologically significant compounds (plants, animals and the environment)	Cyfluthrin, beta-cyfluthrin
Acute toxicity	
Rat, LD_{50}, oral	11 to > 1000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	0.081 to > 1.0 mg/l of air
Rabbit, skin irritation	Not an irritant
Rabbit, eye irritation	Slightly irritating
Guinea-pig, skin sensitization	Not sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Reduced body weight, clinical signs (rats, dogs)
Lowest relevant oral NOAEL	1 mg/kg bw per day (beta-cyfluthrin, rats) 60 ppm, equal to 1.3 mg/kg bw per day (beta-cyfluthrin, dogs)
Lowest relevant dermal NOAEL	340 mg/kg bw per day (cyfluthrin, rats)
Lowest relevant inhalatory NOAEC	0.09 µg/l (cyfluthrin, rats)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weight (rats)
Lowest relevant NOAEL	150 ppm, equal to 6.2 mg/kg bw per day (cyfluthrin, rats)
Carcinogenicity	Not carcinogenic (mice, rats)
Genotoxicity	
	Not genotoxic in vitro or in vivo
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects (rats)
Lowest relevant reproductive NOAEL	450 ppm, equal to 34.7 mg/kg bw per day, i.e. highest dose tested (cyfluthrin, rats)

Developmental target	delayed ossification (rats); increased post-implantation loss (rabbits)
Lowest relevant developmental NOAEL	9.4 mg/kg bw per day (beta-cyfluthrin, rats)
Neurotoxicity/delayed neurotoxicity	
Neurotoxicity	Behavioural effects (increased motility, grooming and digging movements)
Lowest relevant oral NOAEL	1 mg/kg bw (single and repeated dose by gavage, beta-cyfluthrin and cyfluthrin, rats)
Other toxicological studies	
	No data
Medical data	
	Topical skin effect, characterized by a stinging sensation in the affected areas in laboratory workers. Areas most commonly affected were the face, and mucosal tissues.

Summary for cyfluthrin and beta-cyfluthrin

	Value	Study	Safety factor
Group ADI	0–0.04 mg/kg bw	Based on the ARfD	25
Group ARfD	0.04 mg/kg bw	Rat, acute neurotoxicity, beta-cyfluthrin	25

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CYPERMETHRIN
(INCLUDING ALPHA- AND ZETA-CYPERMETHRIN)

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Explanation

Cypermethrin is the International Organization of Standardization (ISO) approved common name for (*RS*)-α-cyano-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2-,2-dimethylcyclopropanecarboxylate. Cypermethrin is a synthetic pyrethroid insecticide containing three chiral centres, giving a racemic mixture of eight isomers comprising four diasterioisomeric pairs. The cypermethrins are alpha-cyano- or type II pyrethroids. Cypermethrin was first evaluated by the 1979 JMPR, when a temporary acceptable daily intake (ADI) was established. New toxicological data were evaluated by the 1981 JMPR and an ADI of 0–0.05 mg/kg bw per day was established. Cypermethrin was reviewed by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR); this review included alpha-cypermethrin and zeta-cypermethrin, which had not previously been considered by the JMPR.

Cypermethrin and alpha-cypermethrin were considered by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1996 and in 2002. In 2002, JECFA established a group ADI of 0–0.02 mg/kg bw, and recommended that JMPR should also consider this approach. The studies submitted to JECFA were available for consideration by the JMPR at its present meeting. Several studies on cypermethrin that were reviewed by JMPR in 1979 and 1981 were not available at the present meeting, but were considered in this evaluation on the basis of the JMPR summaries. The 2006 JMPR was made aware of a study of developmental neurotoxicity with zeta-cypermethrin that had not been submitted before the present meeting. This study was submitted during the meeting, but was not evaluated in detail; however, based on a brief review, the Meeting concluded that this study was not critical for its final conclusion.

For alpha-cypermethrin, the specifications were established by the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS) and published as *WHO specifications and evaluations for public health pesticides: alpha-cypermethrin* (2006).[1]

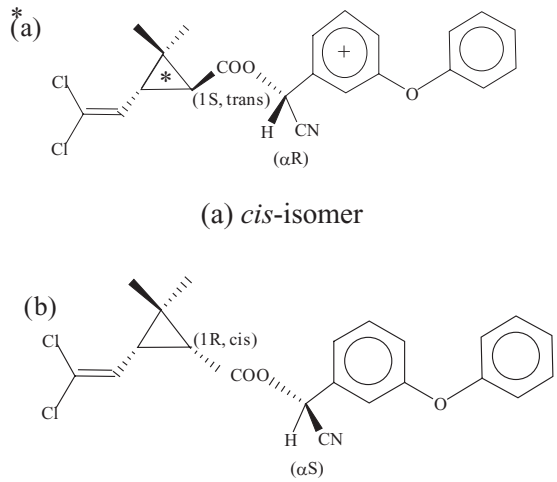
Most studies, excluding those described in previous JMPR monographs, were certified as having been performed in compliance with good laboratory practice (GLP) and in accordance with the relevant Organisation for Economic Co-operation and Development (OECD) test guidelines.

Pyrethroids are typically grouped into two general classes, called type I and type II, on the basis of a combination of toxicological and physical properties. Type II pyrethroids, such as cypermethrin, are those with a cyano group and are characterized by their ability to elicit sinuous writhing (choreoathetosis) and salivation in mammals. A common mode of action has been proposed for pyrethroids, on the basis of in-vitro studies; this includes alterations in the dynamics of sodium channels in tissues of the nervous system, consequent disturbance of membrane polarization, and abnormal discharge in targeted neurons.

As shown in Figure 1, the three chiral centres of cypermethrin are located at carbon positions 1 and 3 of the cyclopropane ring, and at the benzyl α-carbon. The *cis*- and *trans*-configurations each refer to two diasterioisomeric pairs in which the C-1 carboxyl group and the C-3 dichlorovinyl group are on the same, or opposite sides of the cyclopropane ring, respectively. The technical products commonly available contain more than 90% cypermethrin and the ratio of *cis*- to *trans*-isomers varies from 50 : 50 to 40 : 60.

Alpha-cypermethrin contains the most insecticidally active enantiomer pair of the four *cis*-isomers of cypermethrin as a racemic mixture comprising (*R*)-α-cyano-3-phenoxybenzyl (1*S*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and (*S*)-α-cyano-3-phenoxybenzyl (*1R*,3*R*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) (1*R*-*cis*-*S* and 1*S*-*cis*-*R*). In contrast, zeta-cypermethrin is a mixture of the stereoisomers (S)-α-cyano-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate, where the ratio of the (*S*);(1*RS*,3*RS*) isomeric pair to the (*S*);(1*RS*,3*SR*) isomeric pair lies in the range of 45–55 to 55–45 respectively.

Figure 1. Examples of* cis- *and* trans- *isomers of cypermethrin, also showing positions of radiolabels



(a) *cis*-isomer

(b) *trans*-isomer

\+ Benzyl label
* Cyclopropyl label

[1] Available from http://www.who.int/whopes/quality/en/Alphacypermethrin_eval_april_2006.pdf

Evaluation for acceptable daily intake

Unless otherwise stated, the studies reported in this monograph, excluding those assessed in previous JMPR monographs, were certified as having been performed in compliance with good laboratory practice and in accordance with the relevant OECD test guidelines.

Cypermethrin

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

Groups of male mice were given the *cis*- or *trans*-isomers of cypermethrin dissolved in methoxytriglycol as a single oral dose at 7 mg/kg bw as [^{14}C-cyclopropyl]cypermethrin or 8 mg/kg bw as [^{14}C-benzyl]cypermethrin. Results suggest that the proportion of cypermethrin that was readily absorbed after oral dosing was rapidly eliminated in the urine in mice. Absorption from the gastrointestinal tract was more rapid with the *trans*-isomer than with the *cis*-isomer as shown by relatively high concentrations of radioactivity for the *cis*-isomers in the faeces within the first few days of the study. The rate of urinary excretion of cypermethrin isomers labelled in acid or alcohol portions of the molecule was very similar, with most of the radioactivity eliminated from the body within 3 days. Tissue residues after 3 days were low. Adipose tissue was found to retain small quantities of radioactivity in concentrations higher than those noted for most other tissues (Annex 1, references *32*, *33*; Hutson, 1978a).

To evaluate the elimination of cypermethrin and its metabolites from animal tissues after oral administration, groups of male mice were given cypermethrin at doses approximating 9 mg/kg bw as [^{14}C-benzyl]cypermethrin. In contrast to data from other tissues, the rate of elimination of cypermethrin from adipose tissue of mice was relatively slow, with a half-life of approximately 10–20 days. Unchanged *cis*-cypermethrin was the only chemical residue identified in adipose tissue. There was little change in the residue during the last half of the 42-day trial after the relatively rapid elimination during the first 3 weeks, suggesting possible long-term storage and build up of residue in adipose tissue (Annex 1, references *32*, *33*; Crawford & Hutson, 1978a).

Rats

Groups of six male and six female Wistar rats were given cypermethrin (^{14}C-benzyl, *cis*-isomer; radiochemical purity, 99.5%) in corn oil as a single oral dose at 2.4 mg/kg bw for females and 1.8 mg/kg bw for males. Animals and tissues were monitored for 8 days, with two animals of each sex being sacrificed at 24 h, 3 days and 8 days after dosing. Excretion was rapid for both sexes. There was a significant sex difference in the rate of excretion, with male rats excreting a substantially larger portion of the radioactivity in the urine (53%) than females (35%) over the first 24 h, with 19% and 35% of administered radioactivity excreted in the faeces of males and females, respectively, over this period. However, approximately 90% of administered radioactivity was excreted within 48 h in both sexes, with no substantial differences in the overall rate of elimination by males and females when data were evaluated over the total 8-day period of the test. The residues of radioactivity in the tissues of both males and females sacrificed at various periods over the 8 days showed a small residue in a variety of tissues. While the radioactivity in blood, liver, kidney, muscle and brain was rapidly

depleted over the test interval, concentrations of cypermethrin in adipose tissue were relatively stable over the 8 days (Annex 1, reference *33*; Crawford, 1976a).

Groups of three male and three female Wistar rats were given cypermethrin (^{14}C-benzyl, *trans*-isomer) as a single oral dose at 2.4 mg/kg bw for males and 3.0 mg/kg bw for females in corn oil. Again, excretion was rapid in both sexes, with approximately 95% of the administered dose excreted within 48 h. In contrast to the *cis*-isomer, there appeared to be no sex differences in the routes of elimination of the *trans*-isomer. Residues in adipose tissue of females were two to three times higher than in the adipose tissue of males. However, residues in the tissues were lower than after dosing with the *cis*-isomer (Annex 1, reference *33*; Crawford, 1976a). Concentrations in adipose tissue were approximately half those achieved after similar treatment with the *cis*-isomer, suggesting that the *trans*-isomer is metabolized faster than the *cis*-isomer. A low level of administered radioactivity (0.04%) was detected in expired air (Annex 1, reference *33*; Crawford, 1976b).

Groups of three male and three female Wistar rats were given cypermethrin (1 : 1 *cis* : *trans*-isomer ratio, ^{14}C-cyclopropyl, in corn oil) as a single oral dose at a dose of 1 mg/kg bw for males and 2 mg/kg bw for females. The rats were killed after 3 days, and concentrations of radioactivity determined in selected tissues. Rapid elimination of cypermethrin was observed, with a substantial difference noted in 24-h urinary excretion in males and females. Females excreted considerably more radioactivity in the urine (55% of administered radioactivity) over the first 24 h relative to males (32%). After 72 h, total urinary and faecal excretion in both sexes was approximately the same. Small quantities of radioactivity were expired as $^{14}CO_2$ (approximately 0.1% of administered radioactivity), which suggested some metabolic breakdown of the cyclopropyl ring. After 3 days, tissue concentrations in liver, kidney, muscle, brain, blood, skin and remaining carcass were low, but concentrations of radioactivity in the livers of males was approximately threefold that in females, and radioactivity in adipose tissue of females was approximately twice that found in males. Radioactivity in the intestines was approximately three times higher in males than in females, averaging 9% of the administered dose. Otherwise, the highest residual radioactivity was in fat, but this was less than 1% of the administered radioactivity (Annex 1, reference *33*; Crawford, 1977).

Administration of [^{14}C-benzyl]cypermethrin (*cis*-isomer) as a single oral dose at approximately 2.5 mg/kg bw to eight female Wistar rats resulted in a residue of approximately 0.3 ppm in the fat after 8 days. Further studies to 42 days after dosing were performed to evaluate the half-life of the *cis*-isomer in fat and the total elimination pattern. At the end of 42 days, residues were observed in fat which were approximately 10% of the concentrations noted at 8 days. There was a 90% loss of the material from fat over the 8–42 day interval during which timed samples were taken. From these data with rats, a half-life of approximately 20–25 days was estimated with respect to removal of residues from fat after a single oral dose. These half-life values were somewhat longer than those noted with mice (Annex 1, reference *33*; Crawford & Hutson, 1978b).

The fate of orally administered *cis*- and *trans*-isomers of cypermethrin was studied in rats given radiolabelled cypermethrin as one of three forms, i.e. ^{14}C-benzyl; ^{14}C-cyclopropyl and ^{14}C-cyano at doses of 1 to 5 mg/kg bw. Radioactivity derived from the ^{14}C-benzyl and ^{14}C-cyclopropyl labelling was rapidly eliminated, mostly in the urine. Tissue residues were generally very low, e.g. 0.01 µg/g in brain, with the exception of fat (about 1 µg/g). Residues derived from the *cis*-isomer tended to be higher than those derived from the *trans*-isomer. The rate of depletion of the residues derived from [^{14}C-benzyl]*cis*-cypermethrin was rapid (t½ , less than about 1 day) from all tissues except fat (t½, 11–12 days). This residue consisted largely of unchanged *cis*-cypermethrin. The rapid elimination of cypermethrin from rats is due primarily to the efficient cleavage of the ester bond giving rise to

polar metabolites, which are further oxidized and conjugated before excretion (Annex 1, reference *37*; Crawford et al., 1981).

The kinetics of cypermethrin in the blood of groups of five to seven male and female Wistar rats given ^{14}C-benzyl- or ^{14}C-cyclopropyl-labelled cypermethrin (50 : 50 of *cis* : *trans*) as single oral doses at 2 mg/kg bw or 200 mg/kg bw were described in two studies. At the lowest dose (2 mg/kg bw), the half-lives for the elimination of radioactivity from the blood of rats given an oral dose of ^{14}C-labelled cypermethrin were: ^{14}C-benzyl label: males, 2.78 h; females 4.36 h; and ^{14}C-cyclopropyl label: males, 4.3 h; females, 4.74 h. The mean peak blood concentrations of cypermethrin equivalents derived from the benzyl label were 1.8 and 1.4 µg/ml at about 3 h after dosing for male and female rats respectively. The equivalent figures for the cyclopropyl label were 0.7 and 0.6 µg/ml at about 3 and 3–4 h after dosing, respectively. This difference could be attributed to a rapid clearance of the cyclopropyl-labelled chemical from the body or to interanimal variation. The mean peak plasma concentrations of benzyl-labelled cypermethrin were 0.19 and 0.18 µg/ml for male and female rats respectively. The equivalent figures for the cyclopropyl label were 0.04 and 0.02 µg/ml. The difference between the two studies can only be accounted for by interanimal variation with respect to the absorption of cypermethrin.

In studies using the highest dose of 200 mg/kg bw (50 : 50 of *cis* : *trans*), there was a high degree of interanimal variation. Each animal behaved differently from the others, both with respect to the toxic effect of the compound and to the amount of labelled chemical in the blood at any given time. The mean peak blood concentrations of cypermethrin equivalents from the benzyl label were 41.9 and 39.7 µg/ml at about 16 and 23 h after dosing for female and male rats, respectively. The equivalent figures for the cyclopropyl label were 6.7 and 10.3 µg/ml blood at about 8 and 24 h, respectively. The peak plasma concentration of cypermethrin equivalents for the cyclopropyl label for female rats, however, occurred at 24 h and not at 8 h after dosing. The mean peak plasma concentration of cypermethrin for the benzyl label was 5.4 µg/ml for both male and female rats. The equivalent figures for the cyclopropyl label were 0.43 and 0.42 µg/ml for male and female rats respectively (Annex 1, reference *37*; Climie, 1980).

The elimination of radioactivity after a high (200 mg/kg bw) single oral dose of either ^{14}C-benzyl- or ^{14}C-cyclopropyl-labelled cypermethrin (50 : 50 of *cis* : *trans*; purity, > 99%) was studies in groups of male and female rats. The purity of the unlabelled cypermethrin used was 98.4% (also 50 : 50 *cis* : *trans*). The overall excretion of cypermethrin and its metabolites was very rapid, with more than 75% and 85% of the administered dose in the case of males and females, respectively, being eliminated within 3 days. The residues in the tissues at 7 days were low with, on average, less than 1% of the total dose being retained in the whole body. The residues in fat were the highest of all tissues (21 to 14 µg/g), but they were considerably less than 100 times more than the residues found in fat when rats were dosed at about 2 mg/kg bw (Crawford, 1977), suggesting that an appreciable portion of the dose was not absorbed. The presence of a large proportion of the dose as untransformed cypermethrin in the faeces, 26–51%, is a further indication of the poor absorption of this insecticide at this dose (Annex 1, reference *37*; Logan, 1980).

The bioaccumulation of radioactivity was investigated in rats given ^{14}C-benzyl-labelled cypermethrin (50 : 50 of *cis* : *trans*) as 28 consecutive daily oral doses at 2 mg/kg bw per day. Twenty-four hours after the 28th daily dose, the tissue distribution of radioactivity was similar in males and females. The highest mean concentrations of radioactivity were found in fat (4100 and 5100 ng equivalents of ^{14}C-cypermethrin/g tissue in males and females respectively), skin (636 and 712 ng equivalents/g of tissue), gastrointestinal tract (547 and 578 ng equivalents/g tissue), liver (566 and 672 ng equivalents/g tissue), kidney (495 and 535 ng equivalents/g tissue), adrenal glands (913 and 665 ng equivalents/g tissue) and ovaries (710 ng equivalents/g tissue). Low mean concentrations were found

in the heart (67.6 and 88.7 ng equivalents/g tissue in males and females respectively), spleen (64.2 and 63.6 ng equivalents/g tissue), muscle (35.2 and 51.7 ng equivalents/g tissue) and bone (72.8 and 44.4 ng equivalents/g tissue). There was no detectable radioactivity in the brain of female rats and only a very low concentration was detected in brains in males (mean, 13.4 ng equivalents/g tissue). Of the individual organs examined, the highest residual radioactivity was found in the gastrointestinal tract (mean, 6405 ng equivalents), liver (mean, 5820 ng equivalents) and kidney (mean, 795 ng equivalents). The high concentration of radioactivity found in fat, skin, ovaries and adrenal glands is consistent with the lipophilic nature of cypermethrin. The pyrethroid group of insecticides is known to undergo extensive metabolism in mammals (Casida & Ruzo, 1980), resulting in metabolites of a less lipophilic nature that will therefore be more rapidly excreted than the parent compound. The accumulation of radioactivity in the liver and kidney found in this study was consistent with these processes of metabolism and excretion. High concentrations of radioactivity were also found in the gastrointestinal tract, reflecting its role in absorption. Less radioactivity was accumulated in the remaining tissues studied, the lowest concentrations being located in the brain (Annex 1, reference *37*; Hall et al., 1980).

A study of bioaccumulation was undertaken to establish the rate and extent of accumulation of cypermethrin-derived ^{14}C label in a selected range of tissues during a daily oral dosing regime and also the rate of elimination of ^{14}C residues after the attainment of steady-state tissue concentrations. Sixty female rats were dosed orally with ^{14}C-benzyl-labelled cypermethrin (50 : 50 of *cis* : *trans*), at a dose of 2 mg/kg (2 µCi/kg) bw in maize oil (2 ml/kg bw) for up to 70 consecutive days. A group of treated (three) and untreated (one) animals was sacrificed at prescribed intervals during the dosing regime in order to monitor the rate and extent of bioaccumulation of ^{14}C in liver, kidney, adipose tissue, blood (whole blood and plasma), skin and ovaries. The elimination of radioactivity from fat demonstrated biphasic characteristics. This was associated with a rapid elimination of residues of *trans*-cypermethrin coupled with a slower elimination of the *cis*-isomer. These elimination characteristics did not allow a simple half-life value to be calculated for elimination of radioactivity from fat. However, as the relative proportions of *cis*- and *trans*-isomers in fat were monitored from day 70 of the study, it was possible to determine the independent half-lives for both isomers. At the termination of dosing, the relative proportions of *cis*- and *trans*-isomers in fat were 88.25% to 11.75%. The half-lives of elimination of *cis*- and *trans*-cypermethrin from fat were calculated by linear regression analysis to be 18.24 and 3.43 days respectively.

After daily dosing for 70 consecutive days, the extent of bioaccumulation of radioactivity in each tissue analysed was shown to reach a plateau. The extent of accumulation, expressed as µg equivalents of cypermethrin per gram of tissue (as mean ± standard deviation [SD] values from a group of three rats), was: liver, 0.97 ± 0.31; kidneys, 0.65 ± 0.24; fat, 3.91 ± 0 25; blood, 0.35 ± 0.13; plasma, 0.64 ± 0.28; skin, 1.86 ± 0.14; and for each ovary, 0.03 ± 0.01 µg equivalents. Such concentrations of radioactivity in ovaries were similar to background values. In addition, the degree of accumulation of ^{14}C-label in the sciatic nerve was determined by analysing nerves isolated from treated animals given cypermethrin as 56 to 70 daily doses. The extent of accumulation of radioactivity did not exceed 0.05 µg equivalents of cypermethrin per nerve, representing a level of radioactivity similar to the background value. After the termination of dosing, the remaining groups of treated and untreated rats were sacrificed at prescribed intervals for 50 days. Tissue residue concentrations of radioactivity were monitored and the rate of elimination of ^{14}C-label from each tissue was determined. After the cessation of dosing, the liver, kidney and blood ^{14}C-residue concentrations fell rapidly and reached control background levels within 29, 8 and 15 days respectively. With regard to skin, a much slower elimination of radioactivity was observed, with half of the radioactivity being eliminated by 18.7 days after dosing (Annex 1, reference *37*; Jones, 1981).

Male and female Wistar rats were given oral doses of ^{14}C-benzyl cypermethrin or ^{14}C-cyclopropyl cypermethrin in corn oil as a single dose of 200 mg/kg bw or repeated doses of 2 mg/kg bw per day

for up to 70 days. Signs typical of pyrethroid toxicity were observed in rats dosed with 200 mg/kg bw, comprising salivation, hunched back, splayed gait, ataxia, convulsions and hypersensitivity, with the usual time of onset 1.5–2 h after dosing. At 200 mg/kg bw, minimal amounts of $^{14}CO_2$ were detected in the expired air. For males/females treated with 200 mg/kg bw cypermethrin, recovery of administered radioactivity in the urine and faeces, respectively, was 29/33% and 55/59% for the ^{14}C-benzyl label, and 41/56% and 46/34% for the ^{14}C-cyclopropyl label, suggesting that 29–56% of the dose was absorbed in most instances, as most of the ^{14}C in the faeces was present as unchanged cypermethrin. Less than 1% of the administered radioactivity remained in the animals 7 days after dosing. The distribution of radioactivity in the tissues was similar at 7 days after a single dose of 200 mg/kg bw cypermethrin labelled at either position, and 24 h after 28 daily doses of 2 mg/kg bw per day ^{14}C-benzyl-labelled cypermethrin, and was also similar in males and females. The highest concentrations of radioactivity occurred in fat, followed by skin, liver, and kidney, with the concentrations in female gonads also relatively high after either treatment regime, and in the adrenals after repeated dosing at 2 mg/kg bw per day. Seven days after the single dose of ^{14}C-cyclopropyl cypermethrin at 200 mg/kg bw, radioactivity in the liver and intestines of males was approximately three times that of females, and an extremely high level in the spleens of males was not explained (not determined in other groups). In a study of bioaccumulation and elimination in female rats dosed with ^{14}C-benzyl cypermethrin at 2 mg/kg bw per day, radioactivity accumulated rapidly in the liver, kidney, fat, blood, skin and ovaries in the first week of dosing, followed by a slow increase that continued throughout the next 9 weeks of treatment. The concentrations of radioactivity in the plasma, liver and kidneys approximately doubled between 4 and 10 weeks of treatment, but concentrations in fat and skin did not change markedly in this interval (Rhodes et al., 1984).

The distribution, kinetics and excretion of ^{14}C-phenyl cypermethrin at a dose of 3.0 mg/kg bw, diluted with unlabelled alpha-cypermethrin (purity, > 98%), were evaluated in female HAN-Ibm Wistar rats after intravenous administration via the tail vein. Radioactivity excreted in the urine and faeces within 120 h of treatment amounted to 89.7% of the administered dose, comprising 61.3% in the urine and 28.4% biliary excretion into the faeces, with approximately 80% of administered radioactivity excreted in the first 48 h. The half-life of radioactivity in the blood was 5.9 h, with similar half-lives of 4.8–9.9 h in most organs and tissues, the exceptions being fat (half-life, > 24 h) and the skin from the back region (half-life, 12.8 h). After 120 h, liver, ovaries, kidneys, sciatic nerve and skin from the back region retained higher concentrations of radioactivity than blood, but concentrations in these tissues (0.04–0.1 µg/g) were much less than the concentration in fat (1.45 µg/g) (Van Dijk & Burri, 1993).

Groups of male beagle dogs were given ^{14}C-benzyl cypermethrin or the individual ^{14}C-benzyl labelled *cis*- and *trans*-isomers of cypermethrin, orally, at a dose of 2 mg/kg bw. Elimination of radioactivity from all animals was rapid, although differences in data from individual dogs precluded a complete evaluation of the rate of elimination. Differences in the rates of elimination of the individual dogs in the study may have been due to differences in absorption, as cypermethrin was given orally in a capsule with no solvent. Tissue residues observed 4 days after oral administration of cypermethrin were extremely low. Most of the excreted material was found in the faeces (80%) with urine containing only 11% of the administered dose. As with other species, residues of cypermethrin were observed in fat, approximating 2% of the administered dose (this residue was estimated to be 0.3 ppm based upon total adipose tissue of the dog) (Annex 1, reference *33*; Crawford, 1979b).

In a study of excretion in humans, cypermethrin (purity, 98.1–98.5%; *cis* : *trans* 1 : 1) was administered to four male subjects as a single oral dose at 0.25–1.5 mg in corn oil contained in a capsule. The urinary excretion of 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid

(DCVA) was rapid and confined to the first 24 h after dosing. On the basis of the assumption that no *cis* to *trans* isomerization occurs, 78% of the *trans*-isomer and 49% of the *cis*-isomer was excreted as free and conjugated DCVA, and the amounts excreted were dose-related. This study confirmed that, as in other mammals, ester cleavage and elimination of the cyclopropanecarboxylic acid moieties is a major route of cypermethrin metabolism in humans (Eadsforth & Baldwin, 1983).

A study of human absorption and excretion was conducted in which cypermethrin was administered by the oral or dermal route to six human volunteers. Metabolites were measured in the urine that was collected for up to 5 days after dosing. In the oral study, 3.3 mg of cypermethrin (purity, 98.9–99.4%, *cis* : *trans* 1 : 1) was administered undiluted as a single oral dose. Metabolites *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCVA) were recovered in the urine in the ratio of 1 : 2, along with slightly less 3-phenoxybenzoic acid and 3-(4'-hydroxyphenoxy) benzoic acid combined (3PBA + 4OH3PBA). Excretion of DCVA peaked within 4 h of dosing, with derivatives of the phenoxybenzyl moiety peaking later at 4–24 h. The mean elimination half-life of total metabolites was 16.5 h. As estimated from the DCVA recovered, mean oral absorption was 36%. In the dermal study, 31 mg of technical-grade cypermethrin (purity, 91.5%, *cis* : *trans* 56 : 44), mixed with surfactants and wetting agents, and finally diluted to 26 mg/ml in soya bean oil, was applied over a skin area of 800 cm^2 on the backs of the volunteers. A large proportion of the dose was recovered in the wash (approximately 41%, 8 h after application) and from tee-shirts worn overnight (approximately24%). In contrast to the oral study, the *cis* : *trans* ratio for DCVA in the urine was 1.2 : 1, and the amount of 3PBA + 4OH3PBA was approximately fourfold that of DCVA. Urinary excretion of cypermethrin metabolites peaked in the 12–36 h after dosing, with an elimination half-life of approximately13 h. An estimate of the amount of cypermethrin absorbed dermally based on the total DCVA recovered was approximately 0.3%, considerably less than the estimate of approximately 1.2% based on the phenoxybenzyl derivatives. This demonstrates that the use of urinary DCVA in biological monitoring may underestimate dermal absorption (Woollen et al., 1992).

1.2 Biotransformation

The metabolic fate of cypermethrin was investigated in a variety of mammalian species and was determined to be relatively similar in the different species. Studies on the metabolic fate of both *cis*- and *trans*-cypermethrin administered orally to rats have shown that cypermethrin is rapidly cleaved at the ester bond to yield the cyclopropanecarboxylic acid and the 3-phenoxybenzyl alcohol moiety. The latter is rapidly oxidized to 3-phenoxybenzoic acid and conjugated before elimination. Major reactions occurring with the cyclopropanecarboxylic acid include, in part, oxidation at the methyl groups and apparent lactone rearrangement before conjugation and elimination. Hydroxylation of the phenoxybenzoyl moiety has been noted to occur in at least two positions before conjugation and elimination.. Rats and mice metabolized cypermethrin in a similar fashion. In mice, a substantial portion of the phenoxybenzoic acid was conjugated with taurine as well as with glucuronic acid (Annex 1, reference *33*; Hutson, 1977).

Oxidation has been noted to occur in the 4′- position of phenoxybenzoic acid. After oxidation, the molecule is conjugated as a sulfate and excreted. A portion of unconjugated hydroxylated phenoxybenzoic acid was also excreted. Hydroxylated cypermethrin was observed in the faeces in mice, suggesting that hydroxylation may occur before ester cleavage (Annex 1, reference *33*; Hutson, 1978b).

Shono et al. (1978), using microsomal preparations from mouse liver, showed that hydroxylation of the phenoxybenzyl moiety at positions other than the 4′-position can occur. Small amounts of the 5- and 6-hydroxy phenoxybenzyl derivatives were also observed. Both *cis*- and *trans*-isomers were

rapidly metabolized by cleavage of the ester bond and aromatic hydroxylation at the 4′- position of the 3-phenoxybenzyl alcohol moiety.

In rats, approximately half of the administered cypermethrin was excreted as sulfate conjugates of the hydroxylated phenoxybenzoic acid. Phenoxybenzoic acid was also excreted free and conjugated with glycine in contrast to the conjugate pattern noted in mice (Annex 1, reference *33*; Crawford & Hutson, 1977, 1978c).

In studies with cyclopropanecarboxylic acid-labelled cypermethrin, hydroxylation of the methyl groups on the cyclopropane ring occurred to a limited extent. Oxidation was believed to have occurred after ester cleavage. While most of the radioactive metabolites were found in the urine, a small quantity of hydroxylated metabolites were eliminated via the biliary-intestinal-faecal route suggesting absorption and redistribution through the bile to the faeces (Annex 1, reference *33*; Crawford & Hutson, 1978c).

In mice, rats and other species, ester cleavage is rapid. There is quantitative and qualitative evidence to suggest that the *cis*-isomer is more stable, yielding a larger variety of hydroxylated products before ester cleavage, while the *trans*-isomer yields a wider variety of hydrolytic products which in part are oxidized after the ester cleavage. The acid fragment is conjugated predominantly with glucuronic acid as a β-glucuronide. Conjugated cyclopropanecarboxylic acid derivatives in urine identified as glucuronides have a relative resistance to the action of β-glucuronidase. β-Glucuronides formed from these acids are generally of the ester type readily cleaved by mild acid hydrolysis. Thus, treatment of urine with methanol and sulfuric acid will convert both the free acid and the acid glucuronide into a methyl ester. The methyl ester of the carboxylic acid has been shown to be volatile and analytical losses may occur with this compound. The alcohol fragment, the alpha-cyano-3-phenoxybenzyl alcohol, is readily oxidized metabolically to the benzoic acid, conjugated and excreted. Alcohol conjugates with glucuronic acid are generally ether derivatives, stable to acid and enzymatically hydrolysable. The phenoxybenzyl moiety of cypermethrin has been found to form both ester and ether β-glucuronides. Thus, the results of the studies with both rats and mice suggest that most of the excreted metabolites of cypermethrin are hydrolysis products, although hydroxylation of the intact ester has been reported. Hydroxylation of the methyl groups attached to the cyclopropane ring has also been reported. There was no evidence of metabolism at the 2,2-dichlorovinyl moiety. Evidence exists that the *cis*-isomer is somewhat more resistant than the *trans*-isomer to hydrolysis. In all cases, only small amounts of hydroxylated metabolites with the intact ester bond were observed with the cypermethrin.

After administration of a single oral dose of radiolabelled cypermethrin (50 : 50 of *cis* : *trans*) to groups of male and female rats at 200 mg/kg bw, cypermethrin was partially absorbed and rapidly cleaved at the ester bond to produce 2,2-dimethyl-3-(2′,2′-dichlorovinyl) cyclopropanecarboxylic acid and a 3-phenoxy-benzoyl moiety that was mostly further metabolized by oxidation at the 4′- position. The resultant phenol was almost totally conjugated with sulfate, the identical result to that observed in studies at the lowest dose (Crawford & Hutson, 1977). The 4′-hydroxy sulfate forms the major aryl metabolite (16% of the dose) with 3-phenoxybenzoic acid as the second most important (5%). The other identified aryl metabolites are 3-(4-hydroxyphenoxy) benzoic acid (1%) and the glycine conjugate, *N*-(3-phenoxybenzoyl) glycine (1%). The cyclopropyl acid is almost exclusively conjugated as the ester glucuronide (30% of the dose in males, 47% in females) with some free acid (twice as much *trans*- as *cis*-) being found in the urine (4%) together with traces of the *trans*-hydroxymethyl cyclopropyl acids (2%) (Logan, 1980).

Thus, the results of the studies with both rats and mice suggest that most of the excreted metabolites of cypermethrin are hydrolysis products, although hydroxylation of the intact ester has been reported. Hydroxylation of the methyl groups attached to the cyclopropane ring has also been reported. There was no evidence of metabolism at the 2,2-dichlorovinyl moiety. Evidence exists that the *cis*-isomer is somewhat more resistant than the trans-isomer to hydrolysis. In all cases, only small amounts of hydroxylated metabolites with the intact ester bond were observed with the cypermethrin.

The metabolic fate of cypermethrin in dogs was qualitatively similar to that observed with other species with the exception being conjugation reactions of several metabolites. As with other species, cypermethrin is rapidly metabolized by cleavage of the ester bond and by hydroxylation of the phenoxybenzyl moiety at the 4′-position. Sulfate conjugation of the hydroxylated benzoic acid was reported. Additionally, a major urinary metabolite was identified as the 3-phenoxybenzyl glycine. Studies on the metabolic fate of 3-phenoxybenzoic acid in dogs revealed a metabolic pattern of oxidation and conjugation similar to that noted with cypermethrin (Annex 1, reference *33*; Crawford, 1979a, 1979c).

3-Phenoxybenzoic acid has been observed as a major metabolite of cypermethrin in all species studied. Residues of cypermethrin in rat skin (< 3% of the dose) were noted after oral administration. Further studies to define the skin residue were performed using ^{14}C-3-phenoxybenzoic acid administered orally to rats as seven consecutive daily doses. The radioactive metabolites in skin were identified as predominately 3-phenoxybenzoic acid and a small quantity of glyceryl dipalmitate esters of 3-phenoxybenzoic acid (Annex 1, reference *33*; Crawford & Hutson, 1979).

The metabolic fate of cypermethrin in dogs was qualitatively similar to that observed with other species, the exception being conjugation reactions of several metabolites. As with other species, cypermethrin is rapidly metabolized by cleavage of the ester bond and by hydroxylation of the phenoxybenzyl moiety at the 4′-position. Sulfate conjugation of the hydroxylated benzoic acid was reported. Additionally, a major urinary metabolite was identified as 3-phenoxybenzyl glycine. Studies on the metabolic fate of 3-phenoxybenzoic acid in dogs revealed a metabolic pattern of oxidation and conjugation similar to that noted with cypermethrin (Annex 1, reference *33*; Crawford, 1979a, 1979c).

The metabolites derived from a single oral dose of (^{14}C-cyclopropyl) cypermethrin (1 : 1 *cis* : *trans*-WL 43467), administered orally to two male beagle dogs, have been studied. The rapid elimination of cypermethrin in dogs was due to the efficient cleavage of the ester linkage of both the *cis*- and *trans*-isomers and the urinary excretion of the cyclopropanecarboxylic acid moiety, largely as its glucuronic acid conjugate. This metabolite comprised 18% and 51% of the respective doses administered to dog Nos 1 and 2. Absorption of the orally administered cypermethrin was not complete, i.e. 65.6% of the dose was excreted as unchanged cypermethrin in the faeces of dog 1 and 32.1% in the faeces of dog 2. The results of this study confirmed those derived from a similar study in which dogs were dosed with (^{14}C-aryl) cypermethrin, in that most of the excreted metabolites were ester-cleavage products of cypermethrin. While the 3-phenoxybenzyl moiety was mostly hydroxylated in the 4′-position before excretion, most of the cyclopropanecarboxylic acids (*cis*- and *trans*-) were excreted without further metabolism other than glucuronide conjugation. The results of these studies show that the metabolic fate of cypermethrin in dogs is similar to that in rats and mice, but hydroxylation reaction(s) do not occur as readily in dogs as in rats and mice (Annex 1, reference *37*; Crawford & Croucher, 1979).

2. Toxicological studies

2.1 Acute toxicity

(a) Oral, dermal and inhalational toxicity

The acute toxicity of cypermethrin in summarized in Tables 1 and 2.

Cypermethrin-induced signs of toxicity were typical of cyano-containing pyrethroid intoxication. After oral administration, signs of intoxication included sedation, subdued behaviour, ataxia, splayed gait, unsteady gait, tip-toe walking, abasia, diarrhoea, excessive salivation, piloerection, with occasional tremors and clonic convulsions. These signs of toxicity appeared within a few hours after dosing. Survivors generally recovered within several days (Coombs et al., 1976; Freeman, 1987), although clinical signs persisted in some rats throughout the 2-week period of observation after oral or dermal dosing in one study (Henderson & Parkinson, 1980). Acute toxicity after dermal administration is low (Coombs et al., 1976; Jaggers, 1979; Henderson & Parkinson, 1980), with few overt signs of toxicity reported. Immediately after exposure by inhalation, salivation, lachrimation, reduced reflexes, reduced activity, slow deep breathing, shaking and reduced stability were reported, followed by splayed or tiptoe gait and tail erection (Brammer, 1989). In dogs, signs of acute intoxication noted at extremely high doses included nervousness, inappetence, diarrhoea, vomiting, tremors and exaggerated ataxia while walking (Coombs et al., 1976). Studies that indicate that the *cis*-isomer of cypermethrin is more acutely toxic via the oral route in rats than the *trans*-isomer are summarized in Table 2.

Table 1. Results of studies of acute toxicity with cypermethrin[a]

Species	Sex	Strain	Route	Vehicle/comments	LD_{50} (mg/kg bw)/ LC_{50} (mg/m^3)	Reference
Mouse	M, F	NS	Oral	5% in corn oil	82	Coombs et al. (1976)
Mouse	M, F	CD	Oral	5% in corn oil[e]	88	Rose (1982)
Mouse	M, F	NS	Oral	DMSO	138	Coombs et al. (1976)
Mouse	M, F	CD	Oral	40% in DMSO[e]	1126	Rose (1982)
Mouse	M, F	NS	Oral	Aqueous suspension	779[b]	Jaggers (1979)
Mouse	M, F	CD	Oral	50% aqueous suspension[e]	657	Rose (1982)
Mouse	M, F	CD	Dermal	5% in corn oil[e]	> 100	Rose (1982)
Mouse	M, F	NS	Intraperitoneal	Corn oil	485	Coombs et al. (1976)
Rat	M, F	NS	Oral	Corn oil	251–992	Coombs et al. (1976)
Rat	MF	Alderley Park	Oral	Corn oil[j]	247 309	Henderson & Parkinson (1980)
Rat	M, F	Tac:N [SD]fBR	Oral	5% in corn oil[g]	334	Freeman (1987)
Rat	M	NS	Oral	Glycerol formal	200–400	Coombs et al. (1976)
Rat	F	NS	Oral	Glycerol formal	approximately 200	Coombs et al. (1976)
Rat	M	NS	Oral	Aqueous suspension	400–800	Coombs et al. (1976)
Rat	F	NS	Oral	Aqueous suspension	approximately 400	Coombs et al., 1976
Rat	F	NS	Oral	Aqueous suspension	4123[b]	Jaggers (1979)

Rat	M	NS	Oral	Aqueous suspension	3000[c]	Jaggers (1979)
Rat	M, F	Wistar	Oral	50% aqueous suspension[e]	4000	Rose (1982)
Rat	M, F	NS	Oral	DMSO	303	Coombs et al. (1976)
Rat	M, F	Wistar	Oral	40% in DMSO[e]	4000	Rose (1982)
Rat (3 weeks)[d]	M, F	NS	Oral	DMSO	163	Rose & Dewar (1978)
Rat (6 weeks)[d]	M, F	NS	Oral	DMSO	322	Rose & Dewar (1978)
Rat (12 weeks)[d]	M, F	NS	Oral	DMSO	526	Rose & Dewar (1978)
Rat	F	NS	I.p.	Aqueous suspension	> 500[b]	Jaggers (1979)
Rat	M, F	NS	I.p.	Propylene glycol	1000–2000[b]	Jaggers (1979)
Rat	F	NS	Dermal	Undiluted	> 4800[b]	Jaggers (1979)
Rat	M, F	Alderley Park	Dermal	Undiluted	> 4920[j]	Henderson & Parkinson (1980)
Rat	M, F	NS	Dermal	40% in xylene	> 1600	Coombs et al. (1976)
Rat	M, F	Alpk: APfSD	Inhalation (4 h exp.)[h]	MMAD 3.95–5.20 µm; 16.9–28.8% respirable	1260[f]	Brammer (1989)
Rat	MF	NS	Inhalation (4 h exp.)[i]	MMAD 2.3–2.5 µm;	> 1320 2500	Mount (1992)
Syrian hamster	M, F	NS	Oral	Corn oil	> 400	Combs et al. (1976)
Chinese hamster	M, F	NS	Oral	5% in corn oil	203	Coombs et al. (1976)
Guinea-pig	M	NS	Oral	20% in corn oil	approximately 500	Coombs et al. (1976)
Guinea-pig	F	NS	Oral	Corn oil	> 1000	Coombs et al. (1976)
Guinea-pig	M	NS	Oral	Aqueous suspension	> 4000[b]	Jaggers (1979)
Rabbit	F	NS	Oral	Undiluted	> 2400[b]	Jaggers (1979)
Rabbit	F	NS	Dermal	Undiluted	> 2400[b]	Jaggers (1979)
Rabbit	M, F	NZW	Dermal	Undiluted	> 2460[j]	Henderson & Parkinson (1980)
Domestic fowl	M, F	NS	Oral	DMSO	> 2000	Coombs et al. (1976)
Partridge	M, F	NS	Oral	DMSO	> 3000	Coombs et al. (1976)

DMSO, dimethyl sulfoxide; F, female; M, male.

NZW, New Zealand White

[a] Unless specified, all values refer to data generated using cypermethrin (*cis* : *trans* 50 : 50).

[b] Data generated using cypermethrin (*cis* : *trans* 40 : 60).

[c] Data generated using cypermethrin (*cis* : *trans* 53 : 46).

[d] Refers to the age of the rats.

[e] Purity, 98.1%; *cis* : *trans*-isomer ratio 51 : 49.

[f] Technical material, purity, 72.9%; *cis* : *trans*-isomer ratio not stated.

[g] Purity, 93.5%; *cis* : *trans*-isomer ratio not stated.

[h] Nose-only exposure.

[i] Whole-body exposure; purity, 95.7%; *cis* : *trans*-isomer ratio not stated

[j] Purity, 91.5%; cis : *trans*-isomer ratio 53 : 47.

Table 2. Effect of* cis *:* trans *ratio on acute toxicity of cypermethrin

Species	Sex	Strain	Route	Vehicle	*Cis* : *trans* ratio	LD_{50} (mg/kg bw)	Reference
Rat	M, F	?	Oral	DMSO	*Cis* only	160–300	Brown (1979a)
Rat	M, F	?	Oral	DMSO	*Trans* only	> 2000	Brown (1979b)
Rat	F	?	Oral	Corn oil	90 : 10	367	Jaggers (1979)
Rat	F	?	Oral	Corn oil	40 : 60	891	Jaggers (1979)

DMSO, dimethyl sulfoxide; F, female; M, male.

(b) Dermal and ocular irritation

Groups of four male and four female rabbits were given cypermethrin dermally in a 24-h skin test. A single application of cypermethrin was observed to be a moderate irritant to rabbit skin (Annex 1, reference *33*; Coombs et al., 1976).

When 0.1 ml of cypermethrin (purity, 91.5%; *cis* : *trans* ratio, 53 : 47) was applied undiluted to the skin of five Alderley Park albino rats under occlusive dressings as a single application for 24 h, or as five consecutive 24-h applications, no reactions were reported other than transient desquamation in one rat, resolving by 24 h after removal of the dressings. When 0.5 ml of the same test material was applied to the intact or abraded skin of six New Zealand White rabbits for 24 h under occluded conditions, slight to mild erythema was reported for all animals, resolving in all but one animal by 72 h. Transient slight erythema was observed on the abraded skin of one rabbit. Cypermethrin was not an irritant to rat skin, but a slight irritant to rabbit skin (Henderson & Parkinson, 1980).

A single application of undiluted cypermethrin to rabbit eyes produced mild transient conjunctivitis lasting 2 days (Annex 1, reference *33*; Coombs et al., 1976).

A single application of 0.1 ml of undiluted cypermethrin (purity, 91.5%; *cis* : *trans*, 53 : 47) into one eye of each of six New Zealand White rabbits produced slight conjunctival redness, slight chemosis and some discharge that resolved in four of the rabbits by day 7, the final observation day. In another three rabbits treated similarly, but in which the eyes were rinsed shortly after instillation, similar conjunctival effects resolved by day 7 (Henderson & Parkinson, 1980).

(c) Dermal sensitization

Groups of 10 male and 10 female guinea-pigs were used to assess the skin-sensitizing potential of cypermethrin. Two guinea-pigs out of the 20 exposed to cypermethrin showed a positive skin reaction, indicating that cypermethrin may have a weak skin sensitizing potential under the maximization procedure of Magnusson & Kligman (Annex 1, reference *33*; Coombs et al., 1976).

Using the Buehler test, cypermethrin (purity, 91.5%; *cis* : *trans*, 53 : 47) in corn oil was not a sensitizer in guinea-pigs (Henderson & Parkinson, 1980).

2.2 Short-term studies of toxicity

Rats

Groups of rats (12 of each sex per group, 24 of each sex as controls) were fed diets containing cypermethrin at a concentration of 0, 25, 100, 400, or 1600 ppm for 13 weeks. After 2 weeks

exposure at 1600 ppm, both males and females exhibited hypersensitivity and varying signs of ataxia. Mortality was observed with male rats up to the fifth week of feeding after which the surviving animals improved clinically and appeared normal at the end of the study. Reductions in body weight, growth and food intake were also noted at 1600 ppm. At the conclusion of the study, small increases in plasma urea concentration were noted in both sexes. Males had a slight increase in plasma potassium concentration, while females had a slight increase in alkaline phosphatase activity and plasma protein concentrations at 1600 ppm. Haematological abnormalities noted at 1600 ppm after 13 weeks included a reduction in haemoglobin, erythrocyte volume fraction and erythrocyte count in females and a reduction in kaolin-cephalin clotting time in males. Gross and microscopic pathology performed at the conclusion of the study showed axonal breaks and vacuolation in the sciatic nerve of rats at 1600 ppm. This was especially noted with those animals that showed clinical signs of ataxia and died during the course of the study. At the conclusion of the 13-week trial, sciatic nerve lesions were not noted in any surviving animals. An increased kidney weight in males at 400 ppm was observed that was not associated with any clinical or pathological signs of abnormality. The no-observed-adverse-effect level (NOAEL) was 400 ppm, equal to 40 mg/kg bw per day, on the basis of deaths, clinical signs of neurotoxicity, reduced body-weight gain and food consumption, microscopic abnormalities of the sciatic nerve, and changes in a number of haematological and clinical chemistry parameters at 1600 ppm, equivalent to 160 mg/kg bw per day (Annex 1, reference *33*; NOAEL based on the JMPR summary of Hend & Butterworth, 1976).

Groups of 15 male and 15 female SD albino rats were fed diets containing cypermethrin (FMC 45806; purity, 92.6%; *cis* : *trans*, 52 : 48) at a concentration of 0, 150, 500 or 1500 ppm for 90 days. The cypermethrin was dissolved in corn oil before incorporation into the diet. The animals were observed twice per day for mortality and daily for clinical signs. Palpation for internal masses and measurement of body weight and food consumption were performed weekly. All rats were given an ophthalmoscopic examination at the beginning and end of the study. Haematology and clinical chemistry evaluations were performed on 10 rats of each sex per dose at the conclusion of the study, but urine analysis was not conducted. Animals found dead, or killed at study termination were examined for gross pathological effects, selected organs were weighed, and histopathology performed on selected tissues. The mean daily intake by rats in the groups at 0, 150, 500 and 1500 ppm was, respectively, 0, 11.8, 37.2 and 116 mg/kg bw per day for males and 0, 13.5, 45.0 and 132 mg/kg bw per day for females. Additional groups of five rats of each sex per dose were killed after 90 days, livers were removed and hepatic microsomes prepared for the assay of aminopyrine *N*-demethylase (APDM) activity.

Two males and two females in the groups at 1500 ppm were found dead during the first 4 weeks of the study. Clinical signs of toxicological significance that were observed regularly in both sexes at 1500 ppm included staggered gait, splayed hind legs, tremors, shaking, loss of muscle coordination, hypersensitivity, unthrifty, and yellow abdominal staining, with clonic convulsions reported in one female. These signs were not observed at the lower doses. At 1500 ppm, overall body-weight gain was reduced in males and females by approximately 20% and 12% respectively, relative to controls, and was associated with reduced food consumption. Ophthalmoscopic investigations detected no abnormalities.

Table 3. Significant haematological and clinical chemistry findings in a 90-day study in rats fed diets containing cypermethrin

Parameter	Sex	Dietary concentration (ppm)			
		0	150	500	1500
Erythrocytes (10^6/ml)	M	8.78	8.65	8.72	8.32*
	F	8.30	8.23	8.15	7.87*
Haemoglobin (g/dl)	M	17.59	17.43	17.40	17.20
	F	17.08	16.97	16.75	16.20*
Erythrocyte volume fraction (%)	M	46.08	45.71	45.56	44.91*
	F	46.43	45.80	44.77	44.12*
MCH (10^{-12} g)	M	20.07	20.16	19.98	20.71*
	F	20.61	20.62	20.57	20.65
MCV (10^{-15} l)	M	52.2	53.0	52.3	54.2*
	F	55.9	55.6	54.9	56.1
ALT (U/l)	M	32.8	34.5	35.9	37.2
	F	21.9	25.2	22.8	31.9*
SGGT (U/l)	M	0.68	0.66	0.64	0.82*
	F	1.83	1.46	1.73	1.83
Blood urea nitrogen (mg/dl)	M	13.3	13.5	14.4	14.9
	F	13.7	16.7	15.8	20.1*
Albumin (g/dl)	M	3.84	3.82	3.81	3.89
	F	4.07	3.86	3.96	3.73*
Glucose (mg/dl)	M	127	124.6	121.6	110.5*
	F	100.3	107.5	93.5	90.1
Potassium	M	5.07	5.01	4.94	5.07
	F	4.73	4.81	4.89	5.19*
Hepatic APDM	M	52.2	53.4	64.6*	66.8*
(nmol formaldehyde produced/mg protein per 15 min)	F	14.6	20.0	17.2	23.4

From Norvell (1983)

ALT, alanine aminotransferase; APDM, aminopyrine *N*-demethylase; MCH, mean cell haemoglobin; MCV, mean cell volume; SGGT, serum gamma-glutamyl transferase.

* Significantly different from controls ($p < 0.05$).

Male and female rats at 1500 ppm showed slight, but statistically significant reductions in erythrocyte count, erythrocyte volume fraction and/or haemoglobin, accompanied by increases in mean corpuscular haemoglobin and mean corpuscular volume in males (Table 3). Increased alanine aminotransferase (ALT) activity and decreased albumin in females and increased serum gamma-glutamyl transferase (SGGT) activity in males at 1500 ppm were indicative of toxic effects on the liver. Elevated blood urea nitrogen (BUN) and potassium in females at 1500 ppm suggested mild renal toxicity. The findings in liver were supported by increased liver weight relative to body weight in females at 1500 ppm (3.42, 3.47, 3.62, 3.90 (%) at 0, 150, 500 and 1500 ppm, respectively) and in both sexes in the APDM group (10–16% greater than control weights), but macroscopic and microscopic examination revealed no abnormalities in the liver and kidney, or other organs. The small decrease in serum glucose concentrations in males at 1500 ppm was within normal limits for this strain of rat and was not considered to be biologically significant. Mean hepatic APDM activity was increased in males at 1500 and 500 ppm, but this was considered to be an adaptive response rather than a toxicological effect.

The NOAEL in rats was 1500 ppm, equal to 37 mg/kg bw per day in males and 45 mg/kg bw per day in females, on the basis of deaths, clinical signs, decreased body-weight gain and effects on haematological and clinical chemistry parameters at 1500 ppm (Norvell, 1983).

In a short-term study, groups of five male and five female Alpk:APfSD (Wistar-derived) rats were exposed (nose-only) to cypermethrin technical (purity 87.1%, *cis* : *trans* 50.1 : 49.9) at concentrations of 0, 10, 50 or 250 mg/m^3 for 15 days by inhalation for 21 days. The mass median aerodynamic diameter (MMAD) of the aerosol particles in the chamber was 2.6–2.9 μm, with a geometric standard deviation (GSD) of 1.8–2.1 μm. Satellite groups of five males and five females from rats in the control groups and from those receiving the highest dose were retained for 14 days after the exposure period. Concentrations of cypermethrin were determined in the brains of additional control and highest-dose interim-sacrifice groups (five of each sex, killed on day 10) and an additional five of each sex for all doses, killed on day 22. Rats were monitored daily for clinical signs, and detailed clinical examinations and body-weight measurements were made on days 1, 2, 3, 8, 15 and 22, and weekly thereafter for the satellite groups. Food consumption was calculated on a weekly basis. At termination, haematology and clinical chemistry parameters were determined for all animals in the main study and satellite groups, a full postmortem was conducted, and selected organs weighed. Histopathology was restricted to the control group and group at 250 mg/m^3 groups, plus lungs and abnormal tissues from the other groups.

There were no deaths. Immediately after exposure, salivation and lachrimation were observed in rats in the group at 250 mg/m^3, together with decreased activity and tail erection in both sexes, and head/paw flicking, reduced stability and tiptoe gait in females. At 50 mg/m^3, there were sporadic observations of salivation that were probably related to exposure to the test substance. As no other signs were present at this dose, the study author did not regard this as toxicologically relevant. However, given that the highest dose produced salivation and this was not noted in controls, the salivation findings at 50 mg/m^3 should not be dismissed. On non-exposure days, piloerection or ungroomed appearance were noted for some animals exposed at 250 mg/m^3. Slight body-weight loss (< 5%) was observed at 250 mg/m^3 on day 2 in both sexes, and while female body weight recovered after cessation of treatment, male body weights remained below controls (6–8%) for the remainder of the treatment and recovery periods. Food consumption was reduced at 250 mg/m^3 in the first week of treatment (12–15%), but was subsequently similar to controls. Relative to controls, slight (5–7%) but statistically significant reductions in haemoglobin, erythrocyte volume fraction and erythrocyte counts were seen in males and females at 250 mg/m^3, and occasionally at lower doses, but as these changes were small and dose–response relationships were lacking, they were not attributed to treatment. Similarly, there were slight (5–6%) but statistically significant decreases in total protein in males at ≥ 50 mg/m^3, and in females at 250 mg/m^3, the latter accompanied by a decrease in albumin of a similar magnitude. However, a dose–response relationship was not present in males, and taking into account the small differences from control values, these findings were not considered toxicologically significant. Decreases in alanine aminotransferase activity in males at ≥ 50 mg/m^3 (approximately 25%) also lacked a dose–response relationship and were not supported by changes in other liver enzymes or histopathology. Differences in organ weights between control and treated groups that attained statistical significance were generally small (< 10%), lacked dose–response relationships, or were not significant when expressed as a percentage of body weight, and therefore were not attributed to treatment. In addition, other than the observation of red lungs in one male exposed to cypermethrin at 250 mg/m^3, and for which no histopathology was reported, there were no findings at macroscopic or microscopic examination that indicated treatment-related effects. Cypermethrin was detected in the brain of one treated animal killed at day 10 (65 ng/g tissue), but concentrations were below the limit of detection in all other animals. The no-observed-adverse-effect concentration (NOAEC) was 10 mg/m^3 for males and females on the basis of clinical signs of salivation at 50 mg/m^3 (0.05 mg/l) (Parr-Dobrzanski, 1994).

Rabbits

Occluded dermal applications of cypermethrin at a dose of 0, 2, 20 or 200 mg/kg bw in PEG 300 were made to abraded and intact skin of groups of 10 male and 10 female New Zealand White rabbits. The applications were made for 6 h/day, 5 days per week for 3 weeks. No effects were observed on haematology, clinical chemistry or at macroscopic or microscopic examination. Slight to severe skin irritation was observed in rabbits at 200 mg/kg bw and slight to moderate skin irritation was observed in rabbits at 2 or 20 mg/kg bw. Decreased food consumption and body-weight gain were observed in rabbits treated at 200 mg/kg bw. Absolute and relative gonad weights were reduced in male rabbits at 200 mg/kg bw. The NOAEL was 20 mg/kg bw per day on the basis of decreased body-weight gain, and a decrease in testicular weights at 200 mg/kg bw per day (JECFA, 1996; Henderson & Parkinson, 1981).

Dogs

Groups of four male and four female beagle dogs were fed diets containing cypermethrin (1 : 1 *cis* : *trans*-isomeric mixture) at a concentration of 0, 5, 50, 500 or 1500 ppm for 13 weeks. There was no mortality over the course of the study. However, there were significant signs of poisoning observed at 1500 ppm, including diarrhoea, anorexia and tremors as well as ataxia, incoordination and hyperesthesia. On account of these clinical signs, two male and two female dogs at the highest dose had to be sacrificed before the end of the experiment. Minor variations were observed in various haematological parameters, with the kaolin-cephalin clotting time observed to be consistently lower throughout the study in females at 500 ppm. However, because of the lack of a dose–response relationship, the variability of kaolin-cephalin clotting time throughout the study, and the fact that there were no changes in the prothrombin time, this finding was not considered to be relevant. There were no other significant differences with respect to standard haematological or clinical chemistry parameters. Gross examination of kidneys and organs showed no effect on organ weights attributable to the diet. Microscopic examination of tissues and organs revealed a non-specific focal bronchopneumonia in the lungs of the animals surviving at 1500 ppm. There were no compound-related changes and no histological abnormalities in other tissues examined with the exception of a variation in the intensity of the pink colour of the optic disc noted on ophthalmological examination at the conclusion of the study.

Because of the pink coloration observed in the optic disc, a further experiment was undertaken to determine its cause. Two groups of three male dogs were fed diets containing cypermethrin at a concentration of 0 or 500 ppm for 13 weeks. Specific ophthalmological examinations were made to evaluate the degree of coloration of the optic disc. After 13 weeks, there were no consistent differences between the colour of the optic discs of the treated dogs and controls. The NOAEL was 500 ppm, equivalent to 12.5 mg/kg bw per day, on the basis of clinical signs at 1500 ppm (equivalent to 37.5 mg/kg bw per day) (JECFA, 1996; Buckwell & Butterworth, 1977).

Groups of four male and four female beagle dogs received diets containing cypermethrin (FMC 30980 technical; purity, 95.7%) at a concentration of 0, 300, 600, 800 or 1100 ppm for 3 months. The animals were observed twice per day for clinical signs. Detailed physical examinations, and measurement of food consumption and body weight were performed before treatment and weekly thereafter. Haematology and serum chemistry parameters were evaluated before the study, and at 1.5 and 3 months, but urine analysis was not conducted. All animals were killed on days 92–94, selected organs were weighed, and tissues were examined at the macroscopic and microscopic level. The mean test substance consumption for dogs fed diets containing cypermethrin at 300, 600, 800 and 1100 ppm was, respectively, 10.4, 20.7, 24.6 and 37.0 mg/kg bw per day for males and 12.2, 25.4, 34.3 and 45.2 mg/kg bw per day for females.

No dogs died during the study. Slight tremors were observed in one male in each of the groups at 800 and 1100 ppm on days 66/67 and 71/86 respectively. Tremors were observed in another male at 1100 ppm on most days, commencing on day 60. These were described as slight, except for days 76/77 when they reached moderate intensity. Starting on day 49, slight tremors were reported in one female at 1100 ppm on most days for the remainder of the study. Moderate tremors were reported in this female during the detailed physical examinations at weeks 7, 9 and 12. There were no ophthalmic lesions indicative of toxicity attributable to cypermethrin. At 1100 ppm, overall body-weight gain was reduced in males (24% of control values) and females lost weight (−0.2 kg vs 1.3 kg overall weight gain in controls). Females at 800 ppm also showed a net body-weight loss in the first 5 weeks of the study (−0.4 kg vs 0.7 kg weight gain in controls). For the first 3–5 weeks of the study, food consumption was reduced in both sexes at 800 and 1100 ppm. From week 5, diets were supplemented with an additional 400 g of food for one female at 800 ppm and one male at 1100 ppm that had been losing weight. Thereafter, food consumption remained below control levels in males at 800 ppm only. At 1.5 months, erythrocyte volume fraction was decreased, and mean cell haemoglobin concentration (MCHC) increased to a statistically significant extent in 1100 ppm males, along with decreases in erythrocyte numbers and haemoglobin that did not attain statistical significance. Findings were similar at 3 months, but given that comparable differences existed before testing, they were not attributed to treatment. Slightly increased chloride concentrations were reported in females at 800 and 1100 ppm at 1.5 months (110, 111, 112, 113*, 112* mEq/l; *$p < 0.05$, parametric ANOVA) and in females at 1100 ppm at 3 months (115, 115, 117, 118, 119*;*$p < 0.05$). The lack of a dose–response relationship suggests that the findings at 1.5 months were not related to treatment and, given the lack of related findings, the increase at 3 months is unlikely to have biological significance. There was no treatment-related effect on organ weights, and no macroscopic or microscopic lesions were detected.

The NOAEL was 600 ppm, equal to 20.7 mg/kg bw per day in males and 25.4 mg/kg bw per day in females, on the basis of tremors in one male at 24.6 mg/kg bw per day and body-weight loss in females at 34.3 mg/kg bw per day (Daly, 1994).

Groups of four male and four female beagle dogs were fed diets containing cypermethrin (FMC 30980; purity, 95.7%) at a concentration of 0, 100, 200, 600 or 1100 ppm for 12 months. Cypermethrin was incorporated into the diet as a solution in corn oil. Animals were observed twice daily for clinical signs, and detailed physical examinations and measurement of body weight and food consumption were performed before testing and weekly thereafter. Ophthalmoscopic examinations took place at the beginning and end of the study. Haematology and urine analysis testing were performed before treatment and at 3, 6 and 12 months, and evaluation of clinical chemistry parameters before testing and at 6 and 12 months. All animals found dead or killed at the end of the study were subjected to macroscopic examination, and brain, kidneys, liver, testes/epididymides and thyroid/parathyroid were weighed and tissues taken for histopathology. The mean daily intake of cypermethrin in the groups at 100, 200, 600 and 1100 ppm was 2.9, 6.0, 20.4 and 33.9 mg/kg bw per day for males and 3.3, 5.7, 18.1 and 38.1 mg/kg bw per day for females. Two males at 1100 ppm were killed after being found in a moribund condition (days 276 and 324) and one male at 600 ppm was found dead (day 133). Clinical signs in these animals, along with the remaining males and two females at 1100 ppm, and another male at 600 ppm, included irregular gait and whole-body tremors, and/or excessive salivation, prostration, incoordination, decreased activity, clonic convulsions, unthrifty coat and moderate alopecia. There were no treatment-related ophthalmoscopic findings. At 600 ppm, body weights in males were 7–12% less than those of controls from week 19, and approximately 10% less than controls in the second half of the study in females. At 1100 ppm, body weights in males were 4–11% below control values for most of the study, and females weighed 11–24% less than did the controls from week 16. Only body-weight change in females at 1100 ppm reached statistical significance with respect to the controls, mainly in the latter half of the study. Food consumption was decreased by up to 20% in males at 1100 ppm in weeks 1 and 2, and in females at 800 ppm and

1100 ppm in weeks 1–6, with increased food consumption of up to 50% in females at 1100 ppm throughout the last 5 months of exposure. There were no effects on haematology, clinical chemistry and urine analysis parameters and organ weights, and no ophthalmic, gross or microscopic lesions indicative of cypermethrin toxicity.

The NOAEL was 200 ppm, equal to 6.0 mg/kg bw per day in males and 5.7 mg/kg bw per day in females, on the basis of mortality, clinical signs (particularly irregular gait and whole-body tremors), and decreased body weight at 600 ppm and greater (Daly, 1995).

A 2-year feeding study has been carried out in which groups of four male and four female beagle dogs were fed diets containing cypermethrin at 0, 3, 30, 300 and (initially) 1000 ppm. When severe signs of intoxication were observed in the groups at 1000 ppm, this highest dose was reduced to 750 ppm, and when signs of intoxication persisted it was further reduced to 600 ppm. Signs of intoxication consisted of licking and chewing of the paws, a stiff high-stepping gait, whole-body tremors, head shaking, incoordinate ataxia and, in some cases, convulsions. Some dietary inappetence was observed in males fed cypermethrin at 1000 ppm. These signs were observed in the group fed at 1000 ppm and 750 ppm, but not when the dietary concentration of cypermethrin was reduced to 600 ppm. No signs of intoxication were observed in groups of dogs at 3, 30 and 300 ppm. A reduction in body weight was observed in the group of males at the highest dose in the main feeding study. This was considered a consequence of the dietary inappetence observed early in the study when the dogs were fed cypermethrin at 1000 ppm. No effect on body weight (attributable to feeding of the compound) was observed in dogs at 3, 30 and 300 ppm. Significant but small differences between controls and treated dogs were observed randomly throughout the study. Sodium concentrations in the blood plasma of male dogs at 600 ppm showed a significant decrease in some weeks, but were always within the normal physiological range. Three dogs tended to have high alkaline phosphatase activity throughout most weeks of the study; this made values for males at 300 ppm significantly higher than those of the controls. This effect was not observed at 600 ppm. No similar effect was observed in female dogs. This observation was not therefore considered to be of toxicological significance. Overall, no significant differences were detected in clinical chemistry and haematological estimations that could be attributed to feeding with cypermethrin for 2 years. No compound-related pathological abnormalities were found. A small number of minor organ-weight changes were found in intermediate dose groups, but were not considered to be due to treatment.

The NOAEL was 300 ppm, equivalent to 7.5 mg/kg bw per day, on the basis of reduced body weight in males at 600 ppm, equivalent to 15 mg/kg bw per day (Annex 1, reference *37*; Buckwell, 1981).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 70 male and 70 female SPF-Swiss-derived mice received diets containing cypermethrin (purity, 91.5%, *cis* : *trans* 53 : 47; or purity, 94.0–94.2%, *cis* : *trans* 54 : 46) at a concentration of 0 (two groups), 100, 400 or 1600 ppm for up to 101 weeks. Ten mice of each sex were killed after 52 weeks for interim necropsy. Observations included mortality, clinical signs, body-weight gain, food consumption, haematology, clinical chemistry, organ weights, and macroscopy and microscopy. Body-weight gain of both males and females at 1600 ppm was reduced when compared to that in the combined control groups. Several haematological changes, consistent with mild anaemia, were found in the group at 1600 ppm at the interim kill, but not at termination. At interim kill and at termination, an increase in thrombocytosis and absolute and relative liver weight was seen in males at 1600 ppm. An increase in the incidence of benign alveologenic tumours was observed in females at 1600 ppm, but was within the range of incidence for historical controls.

The NOAEL was 400 ppm, equivalent to 60 mg/kg bw per day, on the basis of reduced body-weight gain at 1600 ppm, equivalent to 240 mg/kg bw per day. The NOAEL for carcinogenicity was 1600 ppm, equivalent to 240 mg/kg bw per day, the highest dose tested (JECFA, 1996; Lindsay et al., 1982).

Rats

Groups of 48 male and 48 female rats rats (96 males and 96 females were used as controls) were maintained under specific pathogen-free (SPF) conditions and fed diets containing 1 : 1 *cis* : *trans* cypermethrin at a concentration of 0, 1, 10, 100 and 1000 ppm for 2 years. Chemical analyses were made confirming stability of cypermethrin in the diet over the test interval.

There was no mortality over the course of the study attributable to cypermethrin in the diet. General health and behaviour of the cypermethrin-fed animals was not different from that of the control animals. Body weight and food consumption data showed a reduction in dietary intake and growth in males and females in groups fed at a dietary concentration of 1000 ppm. Gross morphological changes were noted periodically over the course of the study. At 6 months, males at 1000 ppm had a slightly reduced testes weight, which was not seen thereafter. Slight changes in the gross kidney weight at periodic intervals in the study were not accompanied by notable changes in clinical measurements or microscopic findings. The changes in kidney weight were believed to be unrelated to the presence of cypermethrin in the diet. Liver weight increases were noted at 18 months, again in those animals maintained at the highest dose. The only clinical chemistry change noted over the course of the study was a slight decrease in alkaline phosphatase activity in males at 2 years. The decrease in alkaline phosphatase activity was not dose-related and was not noted in females. Minor fluctuations were seen in various haematological parameters over the course of the study but these, too, were not compound-related or statistically significant. These changes are not believed to be related to the presence of cypermethrin in the diet. Gross and microscopic examination of tissues and organs periodically over the course of the study did not suggest adverse somatic effects.

The kidneys of most animals after 12 months showed chronic nephrosis, histologically characterized as tubular dilatations, interstitial chronic inflammatory infiltration and glomerular scarring. In the liver, varying degrees of chronic inflammatory infiltration, hepatocyte vacuolation and bile-duct proliferation were observed. These changes were characteristic of ageing processes and were not attributable to cypermethrin. Dermal ulceration was present in a number of animals over the course of the study. A portion of the sciatic nerve from 12 animals fed cypermethrin at 1000 ppm for 1 year and 12 animals in the control group was histologically examined for signs of neurotoxicity. There were no differences in the incidence of abnormal fibres in the controls and animals fed cypermethrin for 12 months (Trigg et al., 1977). Microscopic examination of the sciatic nerve from animals sacrificed at the conclusion of the study showed a small number of nerve fibres exhibiting slight Wallerian degeneration changes. These degenerative changes increased with age, but did not appear to be dose-related with respect to severity. Thus, cypermethrin did not induce neuropathy clinically nor did it induce histological evidence of nerve degeneration. There did not appear to be a significant increase in the incidence of tumour formation in either males or females over the course of the study. A large number of pituitary adenomas were reported predominantly in females at all doses including controls. This occurrence did not appear to be dose-related as similar events were recorded at all doses.

The NOAEL for toxicity was 100 ppm, equivalent to 5 mg/kg bw per day, on the basis of reduced body weights in both sexes at 1000 ppm, equivalent to 50 mg/kg bw per day. The NOAEL for carcinogenicity was 1000 ppm, equivalent to 50 mg/kg bw per day, the highest concentration tested (Annex 1, reference *33*; McAusland et al., 1978).

Wistar-derived (Alderley Park) rats were fed diets containing cypermethrin (*cis* : *trans* 54 : 46; purity, 88.9–93.1%) at a concentration of 0, 20, 150 or 1500 ppm. Groups of 52 rats of each sex per dose and 12 of each sex per dose (interim kill) were treated for 24 and 12 months respectively, with corresponding groups (two groups of 52 of each sex and two groups of 12 of each sex) receiving the control diet. The group at the highest dose received 1000 ppm for the first 3–6 weeks but as few signs of toxicity were seen, the dose was increased to 1500 ppm. Rats were observed at least daily for clinical signs, with more detailed examinations also performed on each day for the first few weeks, then weekly. Body weight was recorded weekly for 14 weeks, then fortnightly, and food consumption was measured weekly for 13 weeks, at week 16, then every fourth week. Haematology and clinical chemistry parameters (excluding electrolytes) were measured in different animals, comprising 12 rats of each sex per dose before treatment, weeks 4 and 13, then every 13 weeks, and in a further six of each sex per dose at the interim sacrifice. Prothrombin and kaolin-cephalin times were evaluated at 52 and 104 weeks. Urine analysis was performed on the same animals evaluated for clinical chemistry, and at the same times, except that there was no testing of urine between 52 and 104 weeks. Urine was also analysed for cypermethrin metabolites in two or three animals of each sex per dose before treatment, then at 13-week intervals for 52 weeks. Ophthalmological examinations were conducted for 20 rats of each sex at 0 and 1000 ppm at weeks 52 and 104, on the same animals if possible. All animals, including unscheduled deaths, were necropsied, selected organs were weighed (including pituitary gland, but not thyroid) and tissues were taken for pathology. Liver APDM activity was assayed in six males and six females per group at 52 and 104 weeks, and livers were examined by electron microscopy for the quantification of smooth endoplasmic reticulum (SER).

There were no significant differences in survival among the treated groups. At 1000 ppm, clinical signs of frequent face washing, lack of coordination in hind limbs and/or increased sensitivity to sound were observed up to day 6 of dosing. Clinical signs that occurred when this dose was increased to 1500 ppm were described as very mild signs of neurotoxicity, and typical of those associated with pyrethroid poisoning, but no details were provided. Overall body-weight gain was reduced in males and females at 1500 ppm by 12% and 18%, respectively, relative to controls, and was associated with decreased food consumption in males during the first half of the study. At 150 ppm, body-weight gain was less than that of the combined control groups to a statistically significant extent for the most part of weeks 24 to 64 in females, and in week 68 in males. However, these differences were generally too small (4–6%) to be considered biologically significant. There were no treatment-related ophthalmoscopic effects. At 1500 ppm, erythrocyte parameters differed from control values to a statistically significant extent starting at 6 months, but not at all sampling points. These differences were small (< 5%), and within normal limits, so they were not considered to be adverse findings. leukocyte and platelet counts were unaffected. Relative to controls, prothrombin time was increased in males at 1500 ppm at 12 and 24 months (Table 4), but there was no effect on kaolin-cephalin clotting time. Changes in clinical chemistry parameters relative to controls were limited to the groups at 1500 ppm (Table 4). Urea was increased in the first half of the study, particularly in females. Cholesterol and triglycerides were decreased in males mainly early in the study, with similar effects apparent in females from 6 months. As the animals aged, plasma lipid concentrations became very variable, so that no clear effects were apparent at termination. Also at 1500 ppm, urine volume (18 h) was decreased in males relative to controls at 12 months, accompanied by a slight increase in specific gravity and a slight decrease in urinary protein. The cypermethrin metabolite 3-(4'-hydroxyphenoxyl)-benzoic acid was present in the urine of all treated groups, the amount increasing with dose.

Table 4. Significant findings related to haematology and clinical chemistry (n = 12)[a] in rats fed diets containing cypermethrin

Finding	Time-point (weeks)	Sex	Dietary concentration (ppm)				
			0	0	20	150	1500
Prothrombin	104	M	14.4 (6)	15.0 (6)	15.2 (5)	15.0 (1)	15.8* (6)
time (s)		F	14.8 (6)	14.6 (5)	14.4 (5)	14.8 (3)	15.2 (6)
Urea	26	M	43.1	41.2 (9)	44.7	43.1 (11)	49.9*
		F	59.3	51.3	50.4 (11)	56.3	64.9**
Cholesterol	65	M	175	174 (10)	165 (10)	145 (10)	132*
(mg/dl)		F	148 (11)	138 (11)	153	128	105*
Triglycerides	26	M	149	126	152	146	82**
(mg/dl)		F	126	106	110	108	79*

From Milburn et al. (1982a)

F, females; M, male.

* Significantly different from combined control groups, $p < 0.05$ (t-test, two-sided).

** Significantly different from combined control groups, $p < 0.01$ (t-test, two-sided).

[a] The numbers in parentheses represent the number of animals tested when this varied from $n = 12$.

At 1500 ppm, the weights of several organs (adjusted for body weight) differed from controls by > 10% (Table 5). In females, liver and lung weights were increased at 12 months, but were similar to controls at the end of the study. Kidney weights were decreased in both sexes at 24 months, though kidney weight was increased in males at 12 months. Spleen weights were decreased in both sexes at 24 months. As the organ-weight changes at 1500 ppm were not consistent throughout the study, and did not coincide with changes in clinical chemistry parameters, they are not considered to be related to treatment. Also, no treatment-related histopathological findings were detected in these or other organs examined microscopically.

Table 5. Selected organ weights adjusted for body weight (g)[a] in rats fed diets containing cypermethrin

Organ	Time-point (months)	Sex	Body weight (g)				
			Dietary concentration (ppm)				
			0	0	20	150	1500
Liver	12	M	19.7 (12)	19.5 (12)	18.7 (12)	19.9 (10)	20.8 (12)
		F	12.5 (12)	13.3 (12)	13.8 (12)	12.8 (12)	15.1** (12)
Kidney	12	M	3.43 (12)	3.50 (12)	3.45 (12)	3.45 (11)	3.78* (12)
		F	2.36 (10)	2.50 (12)	2.64* (12)	2.52 (11)	2.52 (12)
	24	M	5.14 (27)	4.68 (27)	4.76 (21)	4.89 (27)	4.39 (27)
		F	3.60 (24)	3.51 (22)	3.59 (23)	3.53 (24)	2.98** (27)
Spleen	24	M	1.77 (26)	1.80 (26)	1.79 (26)	1.73 (21)	1.58* (27)
		F	1.32 (24)	1.21 (22)	1.28 (23)	1.29 (23)	1.18 (27)
Lung	12	M	1.87 (12)	1.86 (12)	1.89 (12)	1.85 (10)	1.88 (12)
		F	1.56 (12)	1.52 (12)	1.49 (12)	1.41 (12)	1.36* (12)

From Milburn et al. (1982a)

* Significantly different from combined control groups, $p < 0.05$ (t-test, two-sided).

** Significantly different from combined control groups, $p < 0.01$ (t-test, two-sided).

[a] The numbers in parentheses represent the number of animals tested.

At 1500 ppm, liver APDM activity was increased and hepatic SER was increased in both sexes at 12 and 24 months, and in females at 150 ppm at 24 months (Table 6). These changes were considered to be adaptations, and not of toxicological significance.

Table 6. Liver aminopyrine N-demethylase activity and smooth endoplasmic reticulum proliferation[a] in rats fed diets containing cypermethrin for 2 years

Finding	Sex	Dietary concentration (ppm)				
		0	0	20	150	1500
Aminopyrine *N*-demethylase activity	M	15.3	17.1	17.6 (8)	17.1 (4)	20.9 (7)
(µmol formaldehyde/h per g liver)	F	9.8	11.0 (5)	11.4	10.9	16.1**
Smooth endoplasmic reticulum proliferation§	M	141 (7)	127 (7)	138 (7)	132	156*
	F	104 (7)	119 (5)	113 (5)	135**	137**

From Milburn et al. (1982a)

F, female; M, male.

[a] Means based on six observations per group unless otherwise indicated by the number in parentheses.

[b] Figures represent the number of analysis points in a 320-point analysis grid coinciding with smooth endoplasmic reticulum.

Benign neurinomas of the heart were identified in one, one, and two male rats at 20, 150 and 1500 ppm, respectively. This type of tumour is not normally reported for Wistar rats, but given the flat dose–response relationship over a wide range, it is unlikely that these tumours arose as a result of treatment. Overall, there were no treatment-related neoplastic changes.

The NOAEL was 150 ppm, equivalent to 7.5 mg/kg bw per day, on the basis of clinical signs and decreased body-weight gain at 1500 ppm, equivalent to 75 mg/kg bw per day (Milburn et al., 1982a).

2.4 Genotoxicity

A battery of tests was conducted to determine the genotoxic potential of cypermethrin in vitro and in vivo (Table 7). The weight of evidence of the results indicated that cypermethrin is not genotoxic.

Table 7. Results of studies of genotoxicity with cypermethrin

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation (Ames)	*S. typhimurium* TA1538; *E. coli* WP2 *uvrA*	0–500 µg/plate[a]	NR[c]	Negative	Brooks (1976)
Reverse mutation (Ames)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0–1000 µg/plate[b]	NR[c]	Negative	Suzuki (1977)
Mitotic gene conversion	*S. cerevesiae*	NR[ac]	NR[c]	Negative	Brooks (1976)
Comet assay	Human peripheral lymphocytes	10, 50, 100, 200 µg/ml in DMSO	97.1	Positive[f]	Undeger & Basaran (2005)
In vivo					
Chromosomal aberration	Chinese hamster	0, 20, 40 mg/kg bw per day for 2 days orally in DMSO[d]	NR[c]	Negative	Dean (1977)
Host-mediated assay	Mouse/*S. cerevesiae*	0, 25, 50 mg/kg bw single oral dose; killed 5 h after intraperitoneal injection of suspension of *S. cerevesiae*	NR[c]	Negative	Brooks (1976)

Alkaline Comet assay	*D. melanogaster* larvae (first instar); brain ganglia and anterior mid-gut	0.004–0.5 µg/ml; killed after 96 ± 2 h exposure in the food	98.5	Positive[e]	Mukhopadhyay et al. (2004)

NR, not reported; DMSO, dimethyl sulfoxide.

[a] With and without metabolic activation (preparation from rats).

[b] With and without mouse liver subcellular activation (preparation from six strains of PCB-treated mice)

[c] Summarized from JMPR 1979 evaluation (Annex 1, reference *33*); these details not provided therein.

[d] Positive control, cyclophosphamide at 100 mg/kg bw; negative control, DMSO.

[e] A dose-related increase relative to controls was seen in tail DNA, tail length and tail moment in the single-cell gel electrophoresis (Comet) assay for both tissues examined.

[f] Increased tail length (50 and 200 µg/ml), increased tail intensity (10 and 200 µg/ml) and increased tail moment (200 µg/ml) were observed, but as dose–response relationships were lacking, only the results at 200 µg/ml were considered to significantly increase DNA damage in human lymphocytes in this test.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 30 male and 30 female Wistar rats received diets containing cypermethrin (purity, 98%) at a concentration of 0, 10, 100 or 500 ppm for 5 weeks before mating and then throughout pregnancy and lactation for three successive generations. Two litters were bred per generation. The first litters were discarded at weaning. Males and females from the second litter were randomly selected to breed the next generation. A significant reduction in body-weight gain was seen in the male and female parent rats receiving cypermethrin at 500 ppm in all three generations. This was correlated with a reduction in food consumption. Litter size was reduced at 500 ppm in the F_{1a} litter at birth and after 7 and 21 days. Litter weights were reduced at 500 ppm in the F_{1a} litters on days 7, 14 and 21 of lactation. No other effects on fertility or reproduction parameters were found and no compound-related gross or microscopic pathological findings were noted in any rats.

The NOAEL for parental toxicity was 100 ppm, equivalent to 7.5 mg/kg bw per day, on the basis of reduced body-weight gain and food consumption at 500 ppm, equivalent to 38 mg/kg bw per day. The NOAEL for reproductive toxicity was 100 ppm, equivalent to 7.5 mg/kg bw per day, on the basis of reduced litter size and litter weights at 500 ppm, equivalent to 38 mg/kg bw per day (JECFA, 1996; Hend et al., 1978; Fish, 1979; Thorpe, 1985).

Groups of 15 male and 30 female Wistar (Alderley Park) rats received diets containing cypermethrin (purity, 91.5%, *cis* : *trans* 55 : 45) at a concentration of 0, 50, 150 or 750 ppm for 11–12 weeks before mating, then throughout pregnancy and lactation for three successive generations. Rats in the F_0 group receiving the highest dose were initially treated at 1000 ppm, but due to clinical signs of neurotoxicity in most animals in the first 3 weeks of the study, this was reduced to 750 ppm after 12 weeks. Clinical observations were performed at least daily, and food consumption was recorded weekly. Body weights were recorded weekly in the period before mating and during pregnancy, then every 4 weeks until termination (but weekly during pregnancy). Pup body weights were recorded on postnatal days 0, 4, 10, 21 and 28. Two litters were bred per generation, with males and females from the second litter randomly selected to breed the following generation. Parental males were sacrificed after mating for the second litters of each generation, and parental females were killed after weaning (21 days postpartum, except for the F_{1a} litters that were separated from their mothers after 28 days). Gross necropsies were performed on all parental animals and 50% of each generation of offspring, the remainder being discarded. Selected organs were weighed and tissues taken for histopathology

for premature deaths, 10 male and 25 female F_1 parents, 5 pups of each sex per group in the F_{1b} and F_{2b}, and 10 pups of each sex per group in the F_{3b}.

Several parental deaths occurred in all groups, but aside from one male at 1000 ppm that was killed in extremis on day 9 after showing signs of severe neurological disturbance, they were not attributed to treatment. Clinical signs observed in most F_0 parental animals during the first 3 weeks of exposure at 1000 ppm were high-stepping gait, ataxia/uncoordinated movement, increased sensitivity to sound, piloerection, increased or decreased activity and unsteady gait, with a low incidence of trembling, weak, hunched, salivation, and increased sensitivity to touch. Toxicity in the F_0 and F_2 parental groups was indicated by significant reductions in body-weight gain in the periods before mating of 10–40% for both sexes at ≥ 750 ppm, and at 150 ppm for F_0 females and F_2 males and females (approximately 10%). Relative to controls, food consumption (g per rat) was reduced at 1000/750 ppm in parental males of all generations, in parental females in the F_0 and F_2, and in 150 ppm F_0 females. During pregnancy, body-weight gain in F_{1b} and F_{2b} females at 750 ppm was 10% less than that of controls up to day 14 of gestation and weight gain in the F_{1b}, F_{2b} and F_{3b} litters at 750 ppm was also less, relative to that of controls (approximately 12%). There were no treatment-related effects on fertility, gestation length, live births, litter size or pup survival index. At 750 ppm, a mammary gland adenocarcinoma was observed in one F_1 parental female, but as this is a common neoplasm in this strain of rat, this isolated finding was not attributed to treatment. One abnormal pup was reportedly observed at 750 ppm in both the F_{2b} and F_{3a}, but no details were provided. Three weanlings died or were killed in extremis. The cause of death of a F_{2a} female pup at 750 ppm could not be determined due to cannibalization. One F_{3a} male pup at 750 ppm had severe internal hydrocephalies, but this abnormality was also seen in a control pup and a pup at 150 ppm in the F_{1b}, so was not attributed to treatment. Clinical signs of ataxia were noted for one male F_{1a} pup at 150 ppm, but histopathology indicated congenital lymphoid hypoplasia, and as there were no similar findings at the highest dose, this was not considered treatment-related. Microphthalmia was observed in three pups in the F_{1b} (0, 150, and 750 ppm), and was not seen in subsequent generations, so was not ascribed to treatment. Overall, there were no treatment-related macroscopic or microscopic findings in the parental animals or the F_{2b} pups.

The NOAEL for parental toxicity was 50 ppm, equal to 3.8 mg/kg bw per day, on the basis of reduced body-weight gain at the higher doses. The NOAEL for pup development was 150 ppm, equal to 11 mg/kg bw per day, due to reduced body-weight gain during lactation. There were no reproductive effects at the highest dose of 750 ppm, equal to 56 mg/kg bw per day (Milburn, 1982b).

(b) Dominant lethal studies

Mice

Groups of 12 male mice (36 mice were used as controls) were given cypermethrin (dissolved in dimethyl sulfoxide) as a single dose at f 0, 6.25, 12.5 or 25 mg/kg bw or as five successive daily oral doses at 0, 2.5 and 5.0 mg/kg bw. After dosing, each male was caged with three virgin females for 7 days. The mating procedure was repeated weekly over an interval of 8 weeks in a standard dominant-lethal test. Females mated to males treated with five daily oral doses at 2.5 mg/kg bw and those mated to males receiving a single dose of 12.5 mg/kg bw showed a significant reduction in the incidence of pregnancy during the second and third week respectively of the onset of treatment. This did not occur with other groups receiving higher or lower doses or at other intervals. In females mated to males treated daily with doses of cypermethrin, a significant reduction in fetal implants was observed during the second week of mating. Early fetal deaths were increased in the second week at 5 mg/kg bw. No such increases occurred in any other weekly interval or any other dosed group. Thus, multiple administration of cypermethrin on five successive days induced a significant reduction in fetal implants during the second week of mating and a marginal increase in early fetal deaths at the same time interval.

To evaluate the potential (noted above) for a dominant lethal effect, a second experiment was performed. Groups of 12 male mice (the control group consisted of 36 mice) were given cypermethrin (dissolved in DMSO) as five daily oral doses at 0, 2.5, 5.0, 7.5 or 10 mg/kg bw. After dosing, each male was mated with three virgin females for 4 days and subsequently provided with virgin females every 4 days for 3 weeks. Female mice were examined for evidence of dominant lethality 13 days after mating. In addition, 40 males of proven fertility were treated for five successive days at the same doses, 0, 2.5, 5.0, 7.5 and 10 mg/kg bw. These animals were also mated with four virgin females on four successive days for 3 weeks. Four animals from each group were sacrificed for histological examination of the testes and epididymis on days 1 and 7 after last dose. In contrast to the previous trial, no reduction in fetal implants was noted in any of the animals mated with cypermethrin-treated males. The number of early fetal deaths was marginally increased at the highest dose in the 12–16 days after dosing and in the first 4 days after treatment with 7.5 mg/kg bw in males. In the groups of animals examined histologically, no abnormalities were detected in the testes and epididymis and there were no observable histological differences between any of the test groups and the controls (Annex 1, reference *33*; Dean et al., 1977).

(c) Developmental toxicity

Rats

Groups of 25 pregnant female Sprague-Dawley rats were given cypermethrin at a dose of 0, 17.5, 35 or 70 mg/kg bw per day by gavage in corn oil during days 6 to 15 of gestation. The females were sacrificed on day 21 of gestation for examination of uterine contents, and fetuses were given a gross examination, including skeletal and somatic examinations. One female at 70 mg/kg bw per day was found dead and one female at 70 mg/kg bw per day was killed for ethical reasons following severe convulsions. Eleven out of 25 females in the group at 70 mg/kg bw per day showed neurological disturbances (ataxia, convulsions, hypersensitivity to noise). A dose-related reduction in maternal body-weight gain was observed in the groups at 35 and 70 mg/kg bw per day. There were no indications of any embryotoxic or teratogenic effects.

The NOAEL for maternal toxicity was 17.5 mg/kg bw per day on the basis of reduced body-weight gain at 35 mg/kg bw per day and higher. The NOAEL for developmental toxicity was 70 mg/kg bw per day on the basis of the absence of fetal effects at the highest dose tested (Annex 1, reference *33*; Tesh et al., 1978).

Rabbits

Groups of 20 pregnant rabbits (30 rabbits were used as an additional control group) were given cypermethrin at an oral dose of 0, 3, 10 or 30 mg/kg bw dissolved in corn oil by gavage from day 6 to day 18 of gestation. On day 28 of gestation, the rabbits were sacrificed and examination made of live fetuses, dead fetuses, resorption sites and corpora lutea. Live fetuses were maintained for 24 h to assess viability. Fetuses were also examined for gross somatic and skeletal deformities. There was no significant mortality or differences in weight gain during gestation. There were no significant differences between control and test groups with respect to pregnancy, fetal death or survival. Although a wide range of skeletal and visceral abnormalities were found in the course of the study, there were no differences between control and test groups with respect to abnormalities.

The NOAEL for maternal and developmental toxicity was 30 mg/kg bw per day as there were no treatment-related effects at the highest dose tested (Annex 1, reference *33*; Dix, 1978).

Groups of 20 mated New Zealand White rabbits were given cypermethrin technical (purity, 95.7%) at a daily dose of 0, 100, 450 or 700 mg/kg bw per day as a 50% concentration in corn oil

by gavage for days 7 to 19 of gestation. The dose was varied by adjusting the volume, the control group receiving the same volume of corn oil as the group at the highest dose. Animals were observed daily for clinical signs and body weight was recorded on day 0 of gestation, daily during days 7–19 of gestation, and on days 24 and 29. A set amount of food (approximately 150 g/dam per day) was provided, and usually consumed in its entirety. On day 29, the dams were killed and necropsied. Gravid uterine weights were recorded, uteri were examined, and the weight, sex, external, soft tissue, head and skeletal alterations of fetuses were recorded.

One dam at 700 mg/kg bw per day was killed on day 11 due to swelling, scabbing and severe ulceration of the vaginal area (red fluid-filled cyst-like swelling in the vaginal wall noted at necropsy), and one dam at 450 mg/kg bw per day was killed on day 26 after aborting. One dam in the control group and one dam at the lowest dose died due to dosing accidents. Otherwise, clinical signs likely to be treatment-related comprised ataxia, red staining of the cage pan liner, and decreased faeces in dams receiving 700 mg/kg bw per day, and pink or red staining of the cage pan liner at 450 mg/kg bw per day, although in the absence of other effects at this dose, this was not considered an adverse finding. Maternal body-weight gain at 700 mg/kg bw per day was less than that in the control group for days 7–19 (0.28, 0.27, 0.21, 0.18*g at 0, 100, 450 and 700 mg/kg bw per day respectively; *p = 0.048, Welch trend test). At 700 mg/kg bw per day, persistent truncus arteriosus and abnormal course of the aortic arch, along with additional lobulation of the left lung were observed in one fetus. Another fetus at this dose had misshapen thoracic centrum, lumbar centrum misshapen, absent thoracic transverse process and enlarged thoracic transverse process. As most of these malformations had been seen in historical controls, these single findings were not attributed to treatment. An increase in incompletely ossified hyoid bodies at 700 mg/kg bw per day (fetal incidence, 12.4 %; litter incidence, 44.4%) compared with the control group (4%/20%) was also dismissed, as this is a common skeletal variation in rabbits, and findings were within the range of incidence for historical controls (2.1–14.6%/15.8–66.7%). Overall, there was no evidence of fetal toxicity at any dose.

The NOAEL for maternal toxicity was 450 mg/kg bw per day on the basis of decreased body-weight gain and clinical signs at 700 mg/kg bw per day. The NOAEL for fetal toxicity was 700 mg/kg bw per day, the highest dose tested (Freeman, 1994).

2.6 Special studies

(a) Neurotoxicity

Rats

Groups of 6 or 12 male rats and 6 or 12 female rats were given cypermethrin (purity, 97%) as a single oral dose at 100, 200 or 400 mg/kg bw as a 5% dispersion in corn oil. The observation period was 9 days. The rats were then killed and examined histologically. All rats showed signs of intoxication. At 400 mg/kg bw, within 4 h after dosing, rats developed signs of intoxication, including coarse tremors, spasmodic movements of the body and tail and bleeding from the nose. Tip-toe walking was also seen in some rats. All animals, except one, were killed, owing to the severity of the signs. Histological examination revealed swelling of the myelin sheaths and breaks of some of the axons of the sciatic nerves. At 200 mg/kg bw, similar effects were observed; eight rats of each sex died or were killed within 48 h of dosing. The remaining four rats survived the observation period. At 100 mg/kg bw all animals survived the 9 days. One female out of 12 showed minimal lesions in the sciatic nerve in this group. A no-effect level (NOEL) could not be determined (Annex 1, reference *33*; Carter & Butterworth, 1976).

The aim of the following study was to compare the effects of cypermethrin with permethrin, but only the aspects of the study relevant to cypermethrin are considered here. The neurobehavioural effects

of cypermethrin (purity, 97%; containing approximately equal proportions of *cis*- and *trans*-isomers), were studied in male and female adult Long Evans rats, employing a functional observational battery (FOB) and an automated measure of motor activity. Cypermethrin was administered as a single dose at 0, 20, 60 or 120 mg/kg bw by gavage to groups of eight males and eight females for the FOB, and groups of 12 males and 12 females for the tests for motor activity. For the tests for motor activity, the highest dose was reduced to 100 mg/kg bw owing to lethality in the FOB study. As preliminary studies indicated that different effects were evident at different times after dosing, FOB assessments were made at 1.5 h and 3 h after dosing, as well as immediately before dosing, with motor activity testing performed at 3, 24 and 48 h after treatment. One male and six female rats treated with cypermethrin at 120 mg/kg bw died on the day of dosing, as did two males at 100 mg/kg bw. Treatment with cypermethrin affected gait, muscle function, tone and equilibrium, and produced autonomic signs and burrowing behaviours, with female rats generally being the more sensitive sex. Animals were often observed lying flat in the home cage, with tremors; they displayed excessive lateral head movements on the open field; and profuse salivation was observed. At ≥ 60 mg/kg bw, salivation, choreoathetosis, altered righting reflex, splayed limbs and flattened posture were observed; urination, landing foot-splay and click response were increased; and arousal, grip strengths, touch response and tail-pinch response were decreased. Resistance to removal from the home cage, and gait abnormalities were increased, and total activity counts were decreased at 20 mg/kg bw and above. Effects were generally present in both sexes, and peaked within a few hours of dosing. Notably, salivation and increased removal reactivity (males) peaked at 1.5 h, subsiding by 3 h, at which point motor and sensory effects were the most evident, with motor effects persisting for 1 to 2 days after dosing, particularly in females. A NOEL could not be determined (McDaniel & Moser, 1993).

In a study on the effect of cypermethrin on spontaneous motor function, groups of 8–18 male Long Evans rats were given cypermethrin (49 : 51, *cis* : *trans*-isomer ratio) at a dose of 0.1 to 120 mg/kg bw by gavage. A marked decrease in spontaneous motor function was seen at 40 mg/kg bw. The dose producing the minimum reliably detectable (30%) decrease in activity was estimated to be 10 ± 1.3 mg/kg bw, and the highest no-effect level was 4.3 mg/kg bw (95% confidence limits, 2.0–6.5 mg/kg bw) (Wolansky et al., 2006).

In a paired feeding study to examine the neurotoxicological effects of high dietary concentrations of cypermethrin, groups of 10 male rats were fed diets containing cypermethrin (45 : 55, *cis* : *trans*-isomer ratio) at a concentration of 0, 1250, 2500, or 5000 ppm for 14 days. Growth was monitored and clinical evaluations for adverse behaviour were recorded during the course of the study. Gross pathology on tissues and organs and microscopic examination of the sciatic nerve were performed at the conclusion of the study. At 5000 ppm, mortality was observed with all rats either dying or being sacrificed in a moribund condition within the first week. At 2500 ppm, six out of 10 rats died before the conclusion of the study. There was no mortality in the group at the lowest dose. Clinical signs of neurotoxicity were characterized by an impaired ability to walk and splayed hind limbs. In extreme cases, clinical signs of ataxia and paralysis were reported. Other clinical signs included: hypersensitivity to external stimuli, gross disorientation and convulsions, the latter generally seen at high doses. The neurotoxic signs of poisoning observed in the group at 1250 ppm after day 3 were spontaneously reversed by day 9 when all surviving animals that were initially affected appeared to be normal. Remission of ataxia in the group at 2500 ppm was also noted within 11 days of treatment. Growth reduction was observed in all animals in the study. At the lowest dose, the rate of growth was delayed for the first few days after which time the rate was consistent with that of controls for the remainder of the study. The absolute body weight of the treated animals, however, was significantly lower than that of the controls at the conclusion of the 2-week study. Reduction in body weight was consistent with reduction in food consumption. Ultrastructural changes in the sciatic nerve were

observed at the intermediate and highest dose, although the number of animals examined was small. There was some evidence of axonal damage in the myelinated nerves primarily in the groups at the intermediate and highest dose. Changes in unmyelinated axons were not observed. In general, the histological and ultrastructural changes in the sciatic nerve, accompanied by clinical signs of poisoning, were not readily apparent at low doses. Thus, cypermethrin, at acutely toxic dietary concentrations, induces damage to sciatic nerves, the clinical signs of which may be reversible (Annex 1, reference *33*; Glaister et al., 1977a).

Groups of six male and six female rats (14 males and 14 females in the control group) were fed diets containing cypermethrin *trans*-isomer at a concentration of 0, 30, 100, 300, 1000 or 3000 ppm for 5 weeks. No mortality was observed over the course of the study. Food intake and growth were reduced in the group at the highest dose. Alkaline phosphatase activity was increased at the two higher doses. Minimal changes were observed at the two higher doses, including several haematological parameters (erythrocyte count and haemoglobin concentration). Spleen weights were increased at 1000 ppm and higher and kidney and liver weights were increased at 3000 ppm. Gross and microscopic histopathological examination of several tissues and organs revealed no cypermethrin-related injuries. Special reference was given to the examination of the sciatic nerve, which showed no damage (Annex 1, reference *33*; Hend & Butterworth, 1977a).

Groups of rats (six male and six female rats per group, 10 of each sex were used as controls) were fed diet containing *cis*-isomer at concentrations of 0, 30, 100, 300, 750 and 1500 ppm for 5 weeks following a protocol similar to that described above. Mortality was observed at 1500 ppm at intervals from 4 to 17 days after the start of the experiment, with all animals displaying signs of poisoning by neurotoxicity. At the next lower dose (750 ppm), almost all of the animals showed gross neurotoxic signs of poisoning, abnormal sensitivity to sound and touch and slight cases of ataxia. There was no compound-related mortality at doses of 750 ppm or less. Growth was reduced at 750 ppm over the course of the study. Significant reductions in food intake were also noted at concentrations of 300 ppm and higher at the initial phase of the study. Gross examination of tissues and organs again revealed a reduction in heart, liver, spleen and kidney weights. Adjustment of the data for terminal body weight revealed a statistically significant change in kidney weight at 300 ppm and higher, while liver weight was increased at 750 ppm. Plasma protein concentrations were decreased and urea and potassium concentrations were increased in male rats at 750 ppm. Gross and microscopic examinations of tissues and organs showed substantial degeneration in both the liver and sciatic nerve at 1500 ppm and higher. Liver lesions consisted of a coagulative necrosis of hepatocytes occurring in males and females. The sciatic nerve from both sexes showed swelling and breaks in axons with concomitant myelin degeneration and vacuolation. There were no lesions observed in the brain or the spinal cord (Annex 1, reference *33*; Hend & Butterworth, 1977b).

Groups of 20 male and 20 female rats were fed diets containing cypermethrin (44 : 56 *cis* : *trans* ratio; purity, 92%) at a concentration of 0, 75, 150 or 1500 ppm for 90 days. Random samples were chemically analysed periodically during the course of the study. At the conclusion of the study, four rats of each sex were maintained on control diets for a 1-week recovery period. There was no mortality over the course of the study, although males and females at 1500 ppm had reduced body weight and food consumption in the first month. After the first month, the growth rate was similar to that of controls, although the rats never achieved the weight noted in the other groups. At the conclusion of the study, haematology, clinical chemistry, urine analysis and histopathology were performed on all rats. With the exception of a slight increase in myeloid/erythroid ratio in the bone marrow of female rats fed at a dietary concentration of 1500 ppm, there were no haematological or urine analysis effects. An increased microsomal (SER) oxidative activity was noted as an increased

hepatic APDM activity in males and females at 1500 ppm and in males at 150 ppm. These adaptive changes in the subcellular portions of the liver were substantially reversed within the 4-week recovery period. Increased liver weight was not noted on gross examination at the conclusion of the study. A slight reduction in pituitary weight of males, while statistically significant, was not dose-related. A slight decrease in female kidney weight was dose-dependent and significantly different from control values at the highest dose. The decrease in kidney weight in males was slight and not significantly different from controls in any of the dietary concentrations. Gross and microscopic (including electron microscopic) examinations of tissues and organs showed no significant differences from the observations noted in controls. Examination of the sciatic nerve from animals in the control group and in the group at 1500 ppm showed no changes that could be directly attributable to the presence of cypermethrin in the diet. Seven of the 16 males at 1500 ppm and 2 of 12 male controls showed slight changes in myelin that may or may not have been brought about by histological fixation of the material before examination. The condition of the sciatic nerve of females was similar to that noted in females in the control group. There were no effects noted in unmyelinated axons in males and females. Interim histological examination at 30 days, during which time clinical signs of poisoning were noted, was not performed in this study.

The NOAEL was 150 ppm, equivalent to 150 mg/kg bw per day, on the basis of clinical signs and reduced body-weight gain and food consumption at 1500 ppm, equivalent to 150 mg/kg bw per day (Annex 1, reference *33*; NOAEL based on JMPR summary of Glaister et al., 1977b).

Hamsters

Groups of male and female Syrian hamsters were given cypermethrin at oral doses exceeding the median lethal dose (LD_{50}) in an attempt to define clinical signs of poisoning and to evaluate histological damage to the sciatic nerve. At doses of 794 mg/kg bw and higher, all animals showed clinical signs of poisoning including tremors, abnormal irregular movements, and an unusual gait. As noted with rats, axon and myelin degeneration was noted in all groups treated. The lesions included swelling and breaks in the axons and clumping of myelin (Annex 1, reference *33*; Butterworth & Clark, 1977).

A series of experiments was performed to further evaluate the neurotoxic potential after shorrt-term, oral administration of cypermethrin to Chinese hamsters. Clinical examination, functional testing and enzyme determinations using β-galactosidase and β-glucuronidase were performed. The functional test consists of measuring the mean slip angle where the animal is maintained on an inclined plane that steadily increases its angle until the animal can no longer maintain a stationary position. The average angle of five replicate trials constituted the mean slip angle test.

Groups of 20 male and 20 female Chinese hamsters were given cypermethrin at a oral dose of 40 mg/kg bw followed by a dose of 20 mg/kg bw per day for the following 4 days. Fifteen animals of each sex served as controls. There was extensive weight loss in all dosed groups and some mortality was observed, primarily as a result of the initial administration of 40 mg/kg bw. There was a loss of fur and a dermal ulceration was observed in the early parts of the study. This dermal occurrence was transient, disappearing rapidly after the treatments were concluded. There was significant weight loss in the initial phase of the study. However, after the last dose, the surviving animals rapidly gained weight at a rate consistent with the control animals. There were no effects noted on the mean slip angle experiment and a marginal increase in β-galactosidase activity was observed in peripheral nerve.

A further experiment with five male and five female Chinese hamsters given cypermethrin at a dose of 0, 5, 10 and 20 mg/kg bw per day for 5 days showed no mortality over the course of the study. There was a slower growth of the animals treated at 20 mg/kg bw, which was reversed by the conclusion of the treatment period. Hyperexcitability was noted in one female at the highest dose. There were no notable differences in behaviour in any other animals. There was a significant deficit in the mean slip angle test with females showing an earlier dose-related deficit than noted in males.

The females recovered from this deficit earlier than males, who showed an erratic pattern of recovery. β-Galactosidase activity was increased at all doses 3 weeks after the start of the experiment. This increase was statistically significant at the intermediate and highest doses. Dermal irritation and fur loss was not noted in this experiment.

Sixteen male and sixteen female Chinese hamsters were given cypermethrin as five daily doses at 30 mg/kg bw or a control of dimethyl sulfoxide only. An additional group of eight animals of each sex were given methylmercury at a dose of 7.5 mg/kg bw as five daily doses as a positive control. With cypermethrin, there was no mortality and the rate of weight gain was consistent with that of the controls. There was transient dermal irritation accompanied by skin ulceration in the majority of the animals. This condition disappeared at the conclusion of the treatment interval. One male administered cypermethrin had an unusual gait. There was a slight deficit in the inclined-plane test which was noted in the early parts of the experiment but was absent by the end of the third week. Increases in β-glucuronidase and β-galactosidase activity were evident in peripheral nerve tissue. The Meeting concluded that cypermethrin when administered at high 'subacute' doses, produced changes in the sciatic nerve consistent with Wallerian degeneration. Biochemical changes were evident as increases in β-glucuronidase and β-galactosidase activity and clinically functional deficits were noted (Annex 1, reference *33*; Dewar & Moffett, 1978b).

Hens

In a study of delayed neurotoxicity, six adult domestic hens received cypermethrin at a dose of 1000 mg/kg bw per day in DMSO for 5 days. After 3 weeks, the dosing regime was repeated and a further 3 weeks later the birds were killed. Positive-control (tri-*ortho*-tolyl phosphate) and negative-control (not dosed) groups were used. None of the cypermethrin-treated hens developed any signs of intoxication. Histological examination of the nervous system revealed no lesions. All birds receiving the positive control developed clinical signs of neurological damage within 15 days and became progressively more unsteady and ataxic thereafter. Histological examination of these animals showed lesions in the cerebellum, sciatic nerve and spinal cord, including axonal and myelin degeneration (Annex 1, reference *33*; Owen & Butterworth, 1977).

In a study to determine the LD_{50} and the delayed neurotoxicity of cypermethrin (purity, 87.8%; *cis* : *trans* 53 : 47) in hens, cypermethrin (in corn oil) was given as single gavage doses at 0, 4096, 5120, 6400, 8000 or 10 000 mg/kg bw to groups of five hens in the LD_{50} study and at 0, 500, 2500, 5000 or 10 000 mg/kg bw to groups of 10 hens in the study of neurotoxicity. Ten birds were dosed similarly with tri-*ortho*-cresyl phosphate (TOCP) as the positive control in the study of neurotoxicity. In the study of LD_{50}, hens were observed daily for clinical signs in the 2 weeks after treatment, and body weight was measured weekly (commencing 2 weeks before dosing). In the study of neurotoxicity, hens were assessed daily for ataxia, body weight was measured twice weekly for 3 weeks, all birds were examined post mortem, and the spinal cord and sciatic nerve were examined microscopically. In the study of LD_{50}, there were no mortalities and no treatment-related clinical signs for 14 days after dosing. In the study of neurotoxicity there were no deaths except for one positive control hen that was sacrificed on day 19 after exhibiting severe ataxia. Ataxia was observed in all hens in the positive-control group, but not in any birds treated with cypermethrin. Relative to negative controls, body weights in cypermethrin-treated hens were decreased in the study of LD_{50} at ≥ 5120 mg/kg bw in the first week after dosing, and in all treated groups in the study of neurotoxicity for the first 3 days after dosing, which was associated with reduced food consumption. No abnormalities were observed at macroscopic examination. Microscopic examination revealed a slightly increased incidence of abnormalities of the spinal cord at 5000 and 10 000 mg/kg bw per day relative to controls, which were also slightly more severe in two of the hens at 10 000 mg/kg bw per day, but as no clinical signs of neurotoxicity were observed in the treated hens, it is unlikely that these represented effects

of treatment. Microscopic findings in most hens in the positive-control group were more severe than in the negative-control group. The LD_{50} of cypermethrin in hens was therefore > 10 000 mg/kg bw. There was no evidence of delayed neurotoxicity in cypermethrin-treated hens (Roberts et al., 1981).

(b) Biochemistry and electrophysiology

Preliminary evidence suggested that an increase in the activity of certain lysosomal enzymes in peripheral nerves and deficits in tests of behavioural function could serve as indicators of peripheral nerve damage. During Wallerian degeneration, the activity of enzymes such as β-glucuronidase and β-galactosidase as well as other enzymes in nerve preparations were shown to be increased significantly (Annex 1, reference *33*; Dewar, 1977a).

Groups of male and female rats were given cypermethrin (1 : 1 *cis* : *trans*) at doses ranging from 25 to 200 mg/kg bw day by oral intubation as a 10% w/v solution in DMSO for five consecutive days. A dose-related functional deficit was observed when the mean slip angle test and the landing foot spread test were applied to the animals. The deficit was maximal from 6 to 14 days after the beginning of treatment and complete functional recovery occurred within 4 weeks. Substantial variation in data from the landing foot-spread test was noted. Data were inconsistent over the course of the study. β-Glucuronidase activity was increased in a dose-dependent fashion in both males and females. The results suggest that cypermethrin produced a primary axonal degeneration, readily measurable 28 days after treatment as an increase in β-glucuronidase activity and in deficits in specific behavioural-function testing of rats (Annex 1, reference *33*; Dewar, 1977b).

When further studies were performed on the biochemical indices of nerve degeneration to compare results with known degenerating compounds (methylmercury), it was observed that the changes in β-glucuronidase and β-galactosidase activity were considerably smaller and less reproducible than those obtained after methylmercury poisoning (7.5 mg/kg bw per day for 7 days). Compared with central versus (vs) peripheral nerve damage, there was no evidence that the trigeminal nerve was more sensitive to the effects of cypermethrin than the sciatic and posterior tibial nerves. At near-lethal doses of cypermethrin, biochemical changes in the trigeminal nerve were consistent with those of Wallerian degeneration. The changes were similar qualitatively to those seen with methylmercury, but were quantitatively much less intense (Annex 1, reference *33*; Dewar & Moffett, 1978a).

Electrophysiological studies were performed to determine whether intoxication with cypermethrin given as single dose or over a short period of time produced changes in the conduction velocity of slower fibres in peripheral nerves or alterations in the maximal motor conduction velocity. There was no evidence to suggest that cypermethrin, at doses that induced severe clinical signs of intoxication, including ataxia, had any effect on maximal motor conduction velocity or conduction velocity of the slower motor fibres in peripheral nerves. Doses used in the study ranged from a single dose at 200 mg/kg to seven consecutive doses at 150 mg/kg followed by two doses at 400 mg/kg. At near-lethal doses there were no effects noted on conduction velocity in the slower motor fibres of the sciatic nerve and tail or on the maximal conduction velocity, even in the presence of clinical signs of acute intoxication and at doses at which previous studies had shown functional degeneration. These electrophysiological findings are reflective of motor function which would suggest that the physiological and functional deficits observed as a result of acute intoxication may be primarily sensory in nature (Annex 1, reference *33*; Dewar & Deacon, 1977).

The following experiment in Wistar rats was performed in two phases. The first phase was conducted to determine the time-course for neurochemical changes occurring in the sciatic/posterior

tibial nerve (SPTN), trigeminal nerve and trigeminal ganglion after treatment with cypermethrin for 5 days per week for 4 weeks. Cypermethrin (purity, 98.2%) was given at a dose of 150 mg/kg bw per day in DMSO (reduced to 100 mg/kg bw per day in arachis oil, after 10 doses, because of mortality). Alpha-cypermethrin (purity, 96.6%) was given at a dose of 37.5 mg/kg bw per day in DMSO (also reduced after 10 days to 25 mg/kg bw per day in arachis oil). Five animals of each sex, treated with either cypermethrin or alpha-cypermethrin, were killed at 2, 3, 4, 5, 6, 8, 10 or 12 weeks and examined.

Dosing resulted in the death of 56% of the cypermethrin-treated animals and 21% of the alpha-cypermethrin-treated animals. The most frequent signs of intoxication included abnormal gait, ataxia, lethargy, chromodacryorrhoea, salivation and hypersensitivity to sensory stimuli. The β-glucuronidase and β-galactosidase activities in the SPTN were increased at 5, 6 and 8 weeks, when compared with controls. The increase was maximal after 5 weeks, and after 12 weeks was comparable to that in the controls. No significant enzyme changes were found in the trigeminal ganglia and trigeminal nerve of treated animals.

The second phase was conducted to establish a dose that did not cause peripheral nerve degeneration in the SPTN, trigeminal nerve and ganglia. Groups of 10 malea nd 10 female rats were given cypermethrin at a dose of 37.5, 75 or 150 mg/kg bw per day in DMSO or alpha-cypermethrin at a dose of 10, 20 or 40 mg/kg bw per day in DMSO, on 5 days per week for 4 weeks. A control group of 10 animals was used. Signs of intoxication similar to those reported in the first phase were seen at the highest dose. A large increase in β-glucuronidase and β-galactosidase activities in the SPTN was seen with cypermethrin at 150 mg/kg bw per day and with alpha-cypermethrin at 40 mg/kg bw per day. In the groups given cypermethrin at 75 mg/kg bw per day or alpha-cypermethrin at 20 mg/kg bw per day, a small increase in β-galactosidase activity was found in the distal and proximal sections of the SPTN. The magnitude of the enzyme changes was similar to those in the first phase. Significant enzyme changes were also found in the trigeminal ganglia and to a lesser extent in the trigeminal nerve of the groups given cypermethrin at 75 or 150 mg/kg bw per day and alpha-cypermethrin at 20 or 40 mg/kg bw per day. No peripheral nerve degeneration was observed with cypermethrin at 37.5 mg/kg bw or with alpha-cypermethrin at 10 mg/kg bw (JECFA, 1996; Rose, 1983).

Alpha-cypermethrin

3. Biochemical aspects: absorption, distribution, metabolism and excretion

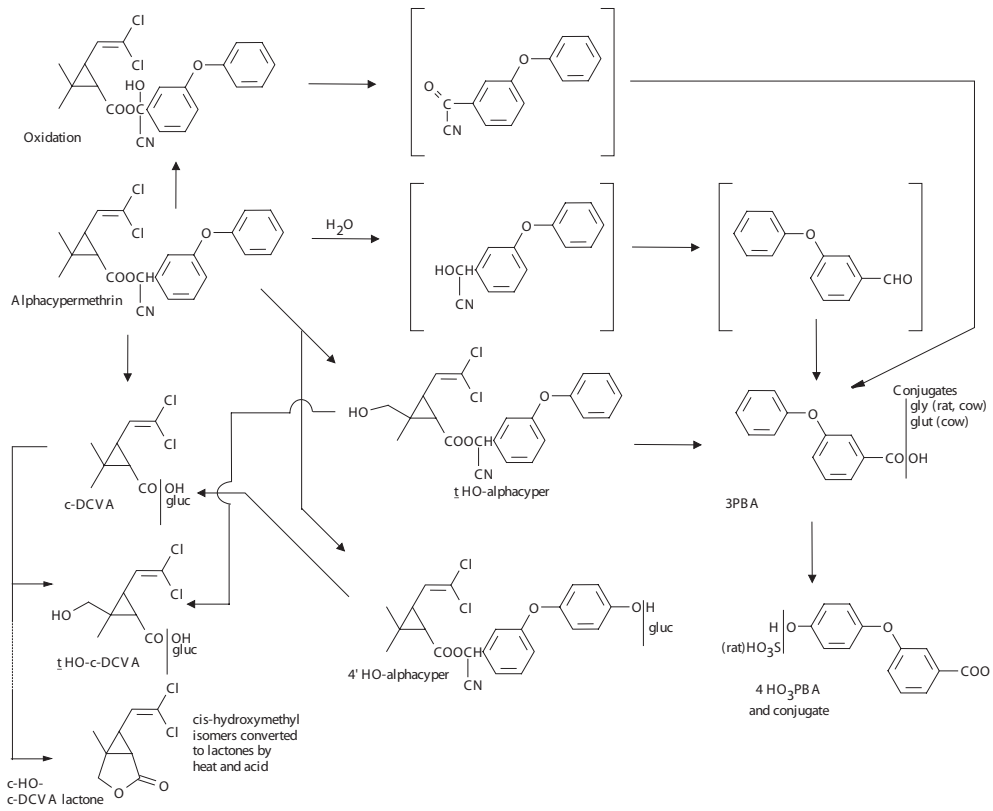
The elimination and retention of alpha-cypermethrin was investigated in male and female Wistar rats. Groups of five male and five female rats received [^{14}C-benzyl]alpha-cypermethrin (radiochemical purity, 99.6%) at a dose of 1.9 mg/kg bw in corn oil by gavage, and were sacrificed 96 h later. The distribution of radioactivity in the excreta was 51–54% in the urine and 38–43% in the faeces. Approximately 75% of the radioactivity in the faeces was unchanged alpha-cypermethrin, with no evidence of conversion from the *cis*-isomer to the *trans*-isomer. Other metabolites identified in faeces were WL 48801 (a dihydroxy metabolite, 4%), 3-(4-hydroxyphenoxy) benzoic acid (6%) and 3-phenoxybenzoic acid (less than 1%). The proposed metabolic pathway for alpha-cypermethrin is provided in Figure 2. More than 75% of the administered radioactivity was excreted within 24 h of treatment. Low concentrations of radioactivity were found in the tissues (total, 1.5% of administered radioactivity). The highest concentration was in fat (0.42 and 0.22 µg/g tissue for males and females, respectively), with the concentration of radioactivity in other tissues at least an order of magnitude less. There were no sex differences (Hutson, 1982).

The depletion of radiolabel from liver, kidney, skin and fat (parovarian, peri-renal and subcutaneous) was studied in 24 female Wistar rats given [^{14}C-benzyl]alpha-cypermethrin

(radiochemical purity, > 99%) as single oral doses at 2 mg/kg bw in corn oil. The concentrations of radioactivity were determined in these tissues at 1, 3, 8, 14, 16, 18, 22 and 42 days after dosing (three animals per time-point). The elimination half-lives in liver and kidney were 2.3 days and 2.0 days respectively. Depletion of radiolabel from fat and skin was biphasic. Elimination half-lives for the initial phase were 1.6–2.5 days, then 17–26 days for the second phase in fat, and 40 days in skin. An analysis of pyrethroids present in the peri-renal and parovarian fat indicated that the administered alpha-cypermethrin had undergone little or no isomerization (Logan, 1983).

In a study in humans, groups of two volunteers received capsules containing alpha-cypermethrin as a single oral doses at 0.25, 0.50 or 0.75 mg (purity, 99.8%) in corn oil. Three weeks later, a repeat-dose trial was performed wherein the same subjects received the same dose as in the first trial, but for five consecutive days. Urine (24 h) samples collected for 4 days after the single dose, and for 10 days after the first of the repeat doses, were analysed for *cis*-cyclopropanecarboxylic acid (free and conjugated). The amount of metabolite excreted varied considerably between individuals, and was related to dose. Excretion was rapid. When expressed as a percentage of the administered dose, the amount of metabolite recovered in the urine was similar for the 24 h period after a single oral dose (range, 35–57%; average, 43%) or any of the repeat doses (range, 30–75%; average 49%), and was fairly consistent over the 5-day dosing period for each individual. Little (1–8% of the administered dose per day) was excreted subsequent to 24 h after dosing. There was no evidence of bioaccumulation in this study (van Sittert et al., 1985).

Figure 2. Metabolic pathways of alpha-cypermethrin, partly gained from analogy with cypermethrin



4. Toxicological studies

4.1 Acute toxicity

The acute toxicity of alpha-cypermethrin is summarized in Table 8.

(a) Oral, dermal and inhalational toxicity

Clinical signs induced by alpha-cypermethrin, like those of cypermethrin, are typical of cyano-containing pyrethroid. The observed signs included ataxia, abasia, gait abnormalities, choreoathetosis, tip-toe walking, increased salivation, lacrimation, piloerection, diarrhoea, unkempt appearance, lethargy, tremor and clonic convulsions. Surviving animals recovered within 9 days (Rose, 1982; Gardner, 1993). Salivation, hypersensitivity to stimuli and hyperactivity were reported in the dermal study in rats (Gardner, 1993).

Table 8. Results of studies of acute toxicity with alpha-cypermethrin

Species	Sex	Strain	Route	Vehicle/comments	LD_{50} (mg/kg bw)/ LC_{50} (mg/m^3)	Reference
Mouse	M, F	CD	Oral	5% in corn oil[a]	35	Rose (1982)
Mouse	M, F	CD	Oral	40% in DMSO[a]	762	Rose (1982)
Mouse	M, F	CD	Oral	50% aqueous suspension[a]	798	Rose (1982)
Mouse	M, F	CD	Dermal	5% in corn oil[a]	> 100	Rose (1982)
Rat	M, F	Wistar	Oral	40% in DMSO[a]	4000	Rose (1982)
Rat	M, F	Wistar	Oral	50% aqueous suspension[a]	> 5000	Rose (1982)
Rat	M, F	Crl:CD BR	Oral	50% aqueous CMC[b]	64	Gardner (1993
Rat	M, F	Crl:CD BR	Dermal	Undiluted technical[b]	> 2000	Gardner (1993
Rat	M, F	SD	inhalation (4 h exp.)[c]	Powder, purity 95.6% MMAD 6.1–9 µm	1590	Jackson (1993

CMC, carboxymethylcellulose; DMSO, dimethyl sulfoxide; MMAD, mass median aerodynamic diameter; SD, Sprague-Dawley.

[a] Purity of test material, 96.6%.

[b] Purity of test material, 95.6%.

[c] Nose-only exposure.

(b) Dermal and ocular irritation

Six New Zealand White rabbits receiving a semi-occlusive topical application of 500 mg of alpha-cypermethrin technical developed very slight erythema that persisted in two animals up to 72 h after removal of the dressings. There were no other dermal reactions (Gardner, 1993).

Six New Zealand White rabbits receiving an instillation of 0.1 ml (equivalent to 45 mg) of alpha-cypermethrin technical showed slight conjunctival redness and ocular discharge at up to 72 h after treatment. No effects on the cornea or iris irritation were observed (Gardner, 1993).

(c) Dermal sensitization

No positive reactions were obtained in a Magnusson & Kligman test performed with guinea-pigs (Gardner, 1993).

4.2 Short-term studies of toxicity

Mice

Groups of eight male and eight female CD-1 mice received diets containing alpha-cypermethrin (purity, 95.4%) at a concentration of 0, 200, 400, 800, 1200 or 1600 mg per kg feed for 29 days, respectively equal to 0, 27, 56, 121, 166 and 241 mg/kg bw per day for males, and 0, 34, 73, 146, 212, and 294 mg/kg bw per day for females. One male at 1600 mg/kg and one female at 1200 mg/kg were killed in a moribund condition. These animals showed neurological disturbances. Mice at 1200 and 1600 mg/kg developed ungroomed coats, ataxia/abnormal gait, over-activity or hunched posture. At 800 mg/kg, some animals had ungroomed coats and abnormal gait. Body-weight gain was dose-relatedly decreased in mice at 1200 and 1600 mg/kg and in females at 800 mg/kg. A similar, but less marked effect was apparent in males receiving 800 mg/kg and females receiving 400 mg/kg. Food consumption was lowered in rats at 1200 and 1600 mg/kg during the first 2 weeks. A depression in lymphocyte numbers was noted in males at 1600 mg/kg. ALT and aspartate aminotransferase (AST) activities were increased in males at 1600 mg/kg. Urea concentrations were increased in all dosed males and females, without a clear dose–response relationship. Plasma albumin and abumin/globulin ratio were decreased in males at 1600 mg/kg. Relative kidney weight was increased in males at 800, 1200 and 1600 mg/kg. Relative lung weight was increased in males at 1600 mg/kg. No macroscopic or microscopic effects were seen. No effects were seen at 400 mg/kg feed, equal to 56 mg/kg bw per day (JECFA, 1996; Green, 1993).

Groups of 12 male and 12 female CD-1 mice received diets containing alpha-cypermethrin (purity, 95.4%) at a concentration of 0, 50, 250 or 1000 mg per kg feed for 13 weeks, equal to 0, 6.3, 33 and 170 mg/kg bw per day for males, and 0, 7.4, 36 and 185 mg/kg bw per day for females. Four males at 1000 mg/kg died during week 12, probably due to stress of treatment and refusal of food and water. The relative kidney weights of these animals appeared to be slightly increased. One mouse at 250 mg/kg died. Clinical signs included thin build, ungroomed coat, hair loss and encrustations of the dorsal body surface in animals receiving 1000 mg/kg, ungroomed haircoat in mice at 250 mg/kg, hair loss and encrustations in males at 250 mg/kg and hair loss in two males at 50 mg/kg, and three males in the control group. Body-weight gain was markedly lower in mice at 1000 mg/kg and lower in mice at 250 mg/kg. Food consumption was slightly higher in mice at 1000 mg/kg. Overall food conversion efficiency of animals at 1000 mg/kg (and to a lesser extent, at 250 mg/kg) was lower than that of controls. In males at 1000 mg/kg, erythrocyte volume fraction, haemoglobin, erythrocyte, total leukocyte and lymphocyte counts were decreased. AST activity was increased in a dose-dependent manner in males at 250 and 1000 mg/kg, and glucose was decreased in males at 1000 mg/kg. Serum alkaline phosphatase activity was increased in females at 1000 mg/kg. Urinary specific gravity was increased in mice at the highest dose. In males at 1000 mg/kg, relative brain, adrenal, heart, kidney, liver, spleen, lung and testes weights were increased. In females, relative liver weight was increased at 250 and 1000 mg/kg and relative brain and spleen weight at 1000 mg/kg. Two males and 11 females at 1000 mg/kg were considered to be emaciated at necropsy. No histological changes were observed.

The NOAEL was 50 mg/kg feed, equal to 6.3 mg/kg bw per day, on the basis of one death, clinical signs, reduced body-weight gain and decreased food conversion efficiency, and increased AST activity in males at 250 mg/kg feed, equal to 33 mg/kg bw per day (JECFA, 1996; modified with reference to the original report of Amyes et al., 1994).

Rats

Groups of 10 male and 10 female Wistar were fed diets containing alpha-cypermethrin (purity, 96.5%) at a concentration of 0 (20 rats of each sex), 20, 100, 200, 400 or 800 mg per kg feed

for 5 weeks. Observations included mortality, clinical signs, body weight and food consumption, haematology, clinical chemistry, urine analysis, organ weight, and macroscopic and microscopic lesions. One male at 800 mg/kg died as a result of factors unrelated to treatment and two males at 800 mg/kg were killed, owing to signs of severe neurological disturbance. Both sexes ar 800 mg/kg showed an abnormal gait and increased sensitivity to noise. Abnormal gait was seen in one male at 400 mg/kg. The mean body weights and food intake of males and females at 400 or 800 mg/kg were significantly lower than those of controls. In males and females at 800 mg/kg and in males at 400 mg/kg, prothrombin time was increased. In males at 800 mg/kg, the kaolin-cephalin coagulation time was increased and the percentage of polymorphic neutrophils was increased at 800 mg/kg. In females, platelet count, total leukocyte count and absolute values of polymorphic neutrophils and lymphocytes were increased, but haemoglobin and erythrocyte volume fraction values were decreased. The MCV was decreased at 200, 400 and 800 mg/kg but not in a dose-related manner. Relative brain, liver and kidney weights were increased in males at 800 mg/kg and relative brain and liver weights were increased in males at 400 mg/kg. In females at 800 mg/kg relative brain, liver and kidney weights were increased. One male at 800 mg/kg that was removed from the study showed scanty axonal lesions of the sciatic nerves.

The NOAEL was 200 mg/kg feed, equivalent to 10 mg/kg bw per day (JECFA, 1996; Thorpe, 1982).

In a dose range-finding study, groups of five male and five female rats (Crl:CD BR) were fed diets containing alpha-cypermethrin (purity, 95.6%) at a concentration of 0, 50, 200, 800 or 1200 mg/kg feed, equivalent to 0, 5, 20, 80 and 120 mg/kg bw per day, for up to 6 weeks. No effects were found on food consumption, haematology, clinical chemistry and microscopy. All rats at 1200 mg/kg and all males at 800 mg/kg were killed during weeks 2 to 4 because of severe clinical signs. These signs included high stepping, splayed gait, abasia and hypersensitivity. Cachexia was seen in extreme cases. There were no significant changes in fore or hind-limb grip strength or hind-limb landing foot-splay. Female rats at 800 mg/kg had a lower mean body weight and food intake compared with controls. These females also had a lower leukocyte count. Microscopic examination revealed lymphocytolysis and lymphocyte depletion of the cortical region of the thymus in males at 800 mg/kg and males and females at 1200 mg/kg. No adverse effects were seen at 200 mg/kg feed, equivalent to 20 mg/kg bw per day. No adverse effects were seen at 200 ppm, equivalent to 20 mg/kg bw per day (JECFA, 1996; Fokkema, 1994a).

Groups of 30 male and 30 female Wistar rats received diets containing alpha-cypermethrin (purity, 94.9%) at a concentration of 0 (60 rats of each sex), 20, 60, 180 or 540 mg per kg feed for 90 days. After 6 weeks, 10 rats of each sex (controls, 20 of each sex) were sacrificed for interim examinations. Three males at 540 mg/kg developed an abnormal gait consisting of splayed hind limbs. Skin sores and fur loss were observed in all males with the highest incidence at 540 mg/kg and in females at 0 and 540 mg/kg. Two males, one from the control group and one at 540 mg/kg were removed because of severe skin lesions. Reduced body weight and reduced food consumption were seen in rats at 540 mg/kg. During the second part of the study, body-weight gain was also reduced in males at 60 and 180 mg/kg, but not in a clearly dose-related manner. Food consumption was lower at 60 mg/kg than at 180 mg/kg. At termination, the haemoglobin value was decreased in males and females at 540 mg/kg. MCV and MCHC were decreased in females at 540 mg/kg. Platelet counts were increased in males and females at 540 mg/kg. The number of lymphocytes was increased and the number of eosinophils was decreased in males at 540 mg/kg. Urea concentration was increased in females at 540 mg/kg. Alkaline phosphatase activity was decreased in females at 180 and 540 mg/kg. Urinary volume was decreased in females at 540 mg/kg and specific gravity was increased in males and females at 540 mg/kg. In females fed 540 mg/kg, relative spleen, heart and brain weights were

increased. In both sexes at 540 mg/kg, relative kidney weight was increased, and relative liver weight was increased in both sexes at 180 and 540 mg/kg. Relative testis weight was increased in males at 540 mg/kg. Histopathological investigations showed a solitary form of axonal degeneration, affecting the fibre only, in the sciatic nerve, in two males fed 540 mg/kg, but there were no clinical signs of toxicity.

The NOAEL was 180 mg/kg feed, equivalent to 18 mg/kg bw per day, on the basis of abnormal gait in males, decreased body-weight gain and food consumption, haematological changes, and axonal degeneration of the sciatic nerve at 540 mg/kg feed, equivalent to 54 mg/kg bw per day (JECFA, 1996; Clark, 1982).

Dogs

This study was conducted in two parts. In the first part, one male and one female beagle dog were fed diets containing alpha-cypermethrin at concentrations of 200 mg/kg feed for 7 days, 400 mg/kg feed for 2 days and 300 mg/kg feed for 7 days. Owing to severe intoxication of the animals at 400 mg/kg, dosing was discontinued after 2 days. The animals were fed the control diet and treatment was restarted at 300 mg/kg in week 3. After the third week, the dogs were killed. Clinical signs including ataxia, body tremors, subdued behaviour, head nodding, food regurgitation, diminished response to stimuli and inflammation of gums and tongue were obtained when dogs were dosed at 300 and 400 mg/kg. Body-weight loss was observed at 300 mg/kg. Examination of the cellular composition of blood and the chemical components of plasma showed no abnormalities, and no macroscopic changes were observed.

In the second part of the study, one male and one female beagle dog were fed diets containing alpha-cypermethrin at a concentration of 300 mg/kg food for 3 days (male dog) or 4 days (female dog), and then 250 mg/kg food for 7 days. At 300 mg/kg, the same effects were obtained as above. At 250 mg/kg, only the female dog developed the clinical signs shown by the dogs treated with 300 and 400 mg/kg (JECFA, 1996; Greenough & Goburdhun, 1984).

Groups of four male and four female beagle dogs received diets containing alpha-cypermethrin (purity, 95.8%) at a dose of 0, 30, 90 or 270 mg (six males and six females) per kg feed for 13 weeks. No effects on mortality, body weight, food consumption, ophthalmoscopy, haematology, clinical chemistry, macroscopy, organ weights or microscopy were seen. One female at 270 mg/kg was killed on day 5 of week 5 because of severe head and body tremors, ataxia, poor limb coordination, inflamed gums and elevated temperature; all dogs at 270 mg/kg developed clinical signs 3–6 h after dosing on days 2 or 3. The clinical signs observed were body tremors and variable incidences of head nodding, lip licking, subdued behaviour, ataxia, and agitation. During week 3, all dogs developed a high-stepping gait and the incidences of the other clinical signs increased as the study progressed. The duration of clinical signs observed early in the study usually resolved within hours after dosing but as the study progressed the duration increased such that they no longer resolved between dosing.

The NOAEL was 90 mg/kg feed, equivalent to 2.25 mg/kg bw per day, on the basis of clinical signs of neurotoxicity at 270 mg/kg feed, equivalent to 6.75 mg/kg bw per day (JECFA, 1996; modified with reference to the original report of Greenough et al., 1984).

Four groups of four male and four female beagle dogs received diets containing alpha-cypermethrin at a concentration of 0, 60, 120 or 240 ppm daily for 52 weeks. No effects were observed on body weight, food consumption, ophthalmoscopy, haematology, clinical chemistry, urine analysis or organ weights, and no changes were observed in macroscopic or microscopic examinations. Two males at 240 ppm developed skin reddening on the tail during week 2 of the study. The tail reddening

caused obvious irritation and resulted in ulceration and necrosis in one male leading to amputation of part of the tail. The tail reddening in the second male resolved after week 8. Abdominal skin reddening and alopecia were seen in a third male at 240 ppm and one female at 120 ppm between weeks 37 to 41.

The NOAEL was 60 ppm, equivalent to 1.5 mg/kg bw per day, on the basis of abdominal skin reddening and alopecia at 120 ppm, equivalent to 3 mg/kg bw per day (JECFA, 1996; modified with reference to the original report of Dean & Jackson, 1995).

4.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of CD-1 mice were fed diets containing alpha-cypermethrin (purity, 95.4%) at a concentration of 0, 30, 100 or 300 ppm for 12 months (20 of each sex) or 18 months (52 of each sex). Animals were observed at least twice daily for mortality and clinical signs. A more detailed examination that included palpation was performed weekly. Food consumption was recorded weekly and body weights were recorded weekly for 14 weeks, and fortnightly thereafter. The only haematological investigations performed were differential leukocyte counts at 50 and 77 weeks. Clinical chemistry and urine analysis were not carried out. Detailed necropsies were performed on all mice found dead or killed in extremis, as well as scheduled deaths at 12 and 18 months. Selected organs were weighed and tissues preserved for histopathology for the groups at 0 and 300 ppm as well as kidneys, liver, and lungs for all mice. The mean daily intake of alpha-cypermethrin in the groups at 30, 100 and 300 ppm was 3.0, 10.6 and 35.2 mg/kg bw per day for males and 3.5, 11.5 and 37.7 for females, respectively.

Survival was not affected by treatment. In males at 300 ppm, the incidences of various clinical signs (thin build, hunched posture, ungroomed coat, hair loss and skin encrustations) were increased, with an increased incidence of ungroomed coat and hair loss in males at 100 ppm also. Mean body-weight gain was significantly decreased by 26% and 24% respectively in males and females in the groups at 300 ppm, and by 13% in males at 100 ppm. Females in the groups at 30 and 100 ppm had mean body-weight gains of 8–11% less than controls, but as these findings did not show a dose–response relationship and lacked statistical significance, they were not considered to be treatment-related. Food consumption in treated and control groups was not significantly different. For the period for which food conversion efficiency was calculated (weeks 1–14), this parameter was reduced in both sexes at 300 ppm, and to a lesser extent in males at 100 ppm. No abnormalities were detected in blood smears, and organ weights were not affected by treatment. The incidence of glandular dysplasia of the stomach was increased to a statistically significant extent in females at 300 ppm (incidence in males/females, 17/6, 9/7, 9/6, 11/15* at 0, 30, 100 and 300 ppm respectively; *$p < 0.05$, Fisher exact test). There were no treatment-related neoplastic findings.

The NOAEL was 30 ppm, equal to 3.5 mg/kg bw per day for males and 3.0 mg/kg bw per day for females, on the basis of decreased body-weight gain at the higher doses (Green, 1996a, 1996b; Cooper, 1999).

4.4 Genotoxicity

The results of assays for genotoxicity with alpha-cypermethrin are summarized in Table 9.

Table 9. Results of studies of genotoxicity with alpha-cypermethrin

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Forward and reverse gene mutation[b, g]	*S. cerevisiae* XV185-14C	31.25–4000 µg/ml in DMSO[a]	95.8	Negative	Brooks (1984)
Reverse mutation (Ames)[f]	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 uvrA	31.5–5000 µg/ml in acetone[a]	95.6	Negative[c]	Brooks & Wiggins (1993a)
Forward mutation[f]	L5178Y mouse lymphoma cells	3.3–50 µg/ml in DMSO[a]	95.4	Negative	Vanderwaart (1994)
Chromosomal aberration[f]	Human peripheral lymphocytes	−S9: 93.75–1000 µg/ml; + S9: 125–1000 µg/ml in acetone	95.6	Negative[d]	Brooks & Wiggins (1993b)
In vivo					
Chromosomal aberration[g]	Rat femoral bone marrow	2–8 mg/kg bw, single oral dose in corn oil[e]	95.8	Negative	Clare & Wiggins (1984)
Micronucleus formation[h]	Mouse	1–10 mg/kg bw single oral dose in corn oil	95.4	Negative	Vanderwaart (1995)
Alkaline elution analysis[g]	Rat liver	40 mg/kg single oral dose in 20% DMSO, 6 h exposure	96.5	Negative	Wooder (1981)

DMSO, dimethylsulfoxide; S9, 9000 × *g* supernatant from livers of male Sprague-Dawley rats.

[a] With and without metabolic activation.

[b] In log- and stationary-phase cultures; positive controls were cyclophosphamide (25 mg/ml in water) and 4-nitroquinoline-*N*-oxide (1 mg/ml in DMSO).

[c] No cytotoxicity.

[d] Precipitation was seen.

[e] Initially doses of 10, 20 or 40 mg/kg were used, but the animals exhibited severe signs of toxicity and the experiment was terminated at these doses. Surviving females were evaluated for chromosomal damage and none was observed.

[f] Two independent experiments performed; positive controls included; GLP and QA statements included.

[g] Positive controls included; QA but no GLP statements included.

[h] Positive controls included; GLP and QA statements included.

4.5 Reproductive toxicity

(a) Developmental toxicity

Rats

In a dose range-finding study, five pregnant female Sprague-Dawley rats received alpha-cypermethrin (purity, 95.6%) at a daily dose of 0, 3, 9, 15 or 18 mg /kg bw in corn oil by gavage during days 6–15 of gestation. Maternal body weights, food consumption and clinical observations were recorded. On day 20 of gestation the females were killed and the fetuses were weighed, sexed and externally examined. Four dams at 18 mg/kg bw per day and one dam at 15 mg/kg bw per day showed hindlimb splay and unsteady gait during dosing. Mean body-weight gain was reduced in a dose-related manner at 9, 15 and 18 mg/kg bw per day and food consumption was reduced in rats at at 15 and 18 mg/kg bw per day. No other treatment-related abnormalities were observed (JECFA, 1996; Irvine & Twomey, 1994).

In the definitive study, groups of 24 pregnant female Sprague-Dawley rats received alpha-cypermethrin (purity, 95.6%) at a dose of 0, 3, 9 or 18 mg/kg bw per day in corn oil by gavage during

days 6–15 of gestation. After marked clinical signs of toxicity, the dose of 18 mg/kg bw per day was lowered to 15 mg/kg bw per day on day 10 of gestation. Clinical signs, body weights and food consumption were recorded. On day 20 of gestation the females were killed and necropsied. The fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities. Females at 18 mg/kg bw per day showed unsteady gait, piloerection, limb splay and hypersensitivity to sound. After reduction of the dose the signs were similar but less marked. After treatment at 18/15 mg/kg bw per day, reduced body-weight gain and food consumption was seen. At 9 mg/kg bw per day, slight body-weight reduction was seen. Mean fetal weights were slightly reduced at 18/15 mg/kg bw per day. There was no indication of teratogenicity.

The NOAEL for maternal and fetal toxicity was 9 mg/kg bw per day on the basis of decreased body-weight gain in the dams, and reduced fetal weights (JECFA, 1996; Irvine, 1994c).

Rabbits

In a dose range finding study, groups of five mated female New Zealand White rabbits received alpha-cypermethrin (purity, 95.6%) at a dose of 0, 5, 15, 25 or 30 mg/kg bw per day as solutions in corn oil by gavage during days 7–19 of gestation. On day 28 of pregnancy the females were killed and a necropsy was performed. The fetuses were weighed, sexed and externally examined. One female each at 15 and 25 mg/kg bw per day was killed prematurely. At 25 and 30 mg/kg bw per day, marked reductions in body weight and food consumption were seen. There was no indication of either embryotoxicity or teratogenicity (JECFA, 1996; Irvine, 1994a).

In the definitive study, groups of 16 pregnant New Zealand White rabbits received alpha-cypermethrin (purity, 95.6%) at a dose of 0, 3, 15 or 30 mg/kg bw per day in corn oil by gavage during days 7–19 of gestation. Maternal clinical signs, body weight and food consumption were recorded. The females were killed on day 28 of pregnancy. The uterus was weighed and the numbers of corpora lutea, implantations and live fetuses were counted. The fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities. Two control females, three at 15 mg/kg bw per day and two at 30 mg/kg bw per day were killed, because of severe weight loss and low food consumption. One female at 15 mg/kg bw aborted on day 28. In all groups, including controls, there was a similar mean body-weight loss after the onset of dosing, which continued until day 11. At 30 mg/kg bw per day, there was a further reduction in mean body-weight gain towards the end of the dosing period. Food consumption reflected the changes in mean body-weight gain.

The NOAEL for maternal toxicity was 15 mg/kg bw per day on the basis of decreased body-weight gain at 30 mg/kg bw per day. The NOAEL for embryotoxicity was 30 mg/kg bw per day, the highest dose tested. There was no indication of teratogenicity (JECFA, 1996; modified with reference to the original report of Irvine, 1994b).

4.6 Special studies

(a) Neurotoxicity

Rats

The acute neurotoxicity of alpha-cypermethrin was studied in Crl:CD:BR rats in two separate studies, each using four groups of 10 males and 10 females or 5 males and 5 females (additional study). The groups received alpha-cypermethrin (purity, 95.6%) as a single dose at 0, 4, 20 or 40 mg/kg bw in corn oil. During the 14-day observation period, clinical signs and body weight were analysed. In the main study, a detailed clinical assessment for neurotoxicological effects was performed. This included a FOB and measurements of fore- and hindlimb grip strength, hindlimb landing foot-splay

and motor activity. In each study, five rats of each sex were killed on day 15 and brain, eyes, muscle, nerves, spinal cord and spinal ganglia were analysed.

One male rat in each of the groups at 20 and 40 mg/kg bw of the additional study was found dead on the day after dosing. Clinical signs were seen in male rats dosed at 20 and 40 mg/kg bw. The signs (similar in both studies) developed between 3 to 8 h after dosing and resolved by 3 days after dosing. The signs included abnormal/splayed gait, thrashing, prostration, vocalization, piloerection, hunched posture, unkempt appearance, soiled/stained body areas and diarrhoea. The signs in females were similar but lower in frequency. In addition to these signs there were also isolated cases of twitching, tremors, abasia, hypersensitivity, pale eyes, soft faeces and thinning of the fur. During the FOB conducted 5 h after dosing, gait abnormalities and clinical signs of increased reactivity were seen in most male rats at 20 and 40 mg/kg bw. In females the signs were less frequent. In the groups at 20 and 40 mg/kg bw there was an increase in very slight to slight sporadic fibre degeneration in the sciatic nerve. The changes were more frequent in the proximal than in the distal part of the nerve.

The NOAEL was 4 mg/kg bw on the basis of death, clinical signs, findings in the FOB and degenerative changes to the sciatic nerve at doses of 20 mg/kg bw and higher (JECFA, 1996; Fokkema, 1994b).

Zeta-cypermethrin

5. Toxicological studies

5.1 Acute toxicity

(a) Oral and dermal toxicity

The acute toxicity of zeta-cypermethrin is summarized in Table 10.

Clinical signs induced by zeta-cypermethrin were similar to those induced by cypermethrin, including tremors, staggered gait, writhing, abnormal posture, squinting eyes, walking on toes, splayed hindlimbs and clonic convulsions, mainly confined to the first 24 h after dosing (Freeman, 1999a; Freeman, 1999b)

Table 10. Studies of acute toxicity with zeta-cypermethrin

Species	Sex	Strain	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw)	Reference
Rat	M	SD	Oral	5% in corn oil	88.2	134	Freeman (1989)
	F					86	
Rat	M	SD	Oral	5% in corn oil	91.2	257	Freeman (1999a)
	F					367	
Rat	M	SD	Oral	5% in corn oil	86.0	269	Freeman (1999b)
	F					285	
Rabbit	M	NZW	Dermal	Undiluted	88.2	> 2000	Freeman (1990b)
	F						

F, female; M, male; NZW, New Zealand White.

(b) Dermal and ocular irritation

After the application of zeta-cypermethrin technical (0.5 ml; purity, 88.2%) to the intact skin of New Zealand White rabbits (three of each sex) for 4 h under semi-occlusive dressings, erythema

was observed in two males at 72 h (Draize score, 1–2), but not before, resolving by day 4 or 6. Zeta-cypermethrin was a slight irritant to rabbit skin in this test (Freeman, 1990d).

After instillation of 0.1 ml of zeta-cypermethrin (purity, 88.2%) into one eye of each of nine New Zealand White rabbits (eyes of three rabbits were washed out with water 20–30 s after treatment), conjunctival redness was present at 1 h, along with chemosis and discharge, persisting until 48 h, but resolving in all except one animal in the washed-out group by day 4. Otherwise, results for washed and unwashed eyes were similar. Corneal opacity and iritis were not observed (Freeman, 1990c).

(c) Dermal sensitization

Zeta-cypermethrin (purity, 88.2%) produced positive reactions in the Buehler test. Slight erythema (Draize score, 1) was observed at 24 h in 9 out of 10 guinea-pigs, and mild erythema (Draize score, 2) was seen in one animal, which also had a scab at the test site. Slight erythema persisted in five animals at 48 h (Freeman, 1990e).

5.2 Short-term studies of toxicity

Rats

In a dose range-finding study, groups of five male and female Fischer 344 rats received diets containing zeta-cypermethrin (purity, 88.2%) at a concentration of 0, 50, 300, 600, 900 or 1500 ppm for 28 days. Animals were observed twice daily for mortality, once daily for clinical signs, and body weight and food consumption were measured weekly. All animals were necropsied and brain, liver, kidney and testes were weighed. Histopathology was not performed. The mean test substance consumption for rats fed zeta-cypermethrin at 50, 100, 300, 600, 900 and 1500 ppm was, respectively, 4, 9, 26, 48, 69 and 105 mg/kg bw per day for males and 5, 10, 27, 52, 74 and 102 mg/kg bw per day for females. All rats at 1500 ppm died between days 7 and 9. Body-weight gain was reduced by 94% in males at 900 ppm and by 18% at 600 ppm. Females at 900 ppm lost weight (–1.8 g compared with a mean gain of 40.6 g in the controls). Food consumption at 900 ppm was reduced by 35% in males and by 28% in females. Treatment-related clinical signs were confined to the groups at 900 ppm, and comprised abdominal staining, ataxia, chromorhinorrhea, decreased faeces, dehydration, splayed hind limbs and unthriftiness. Tremors, convulsions and hypersensitivity to touch were observed in some rats at 1500 ppm before death. The absolute and relative weights of the selected organs were not affected in males, but in females absolute, but not relative, liver weight was reduced compared with controls. No treatment-related gross pathology was present at necropsy (Freeman, 1990a).

Groups of 10 male and 10 female Fischer 344 rats were given diets containing zeta-cypermethrin (purity, 88.2%) at a concentration of 0, 10, 50, 150, 250, 500 or 900 ppm for 90 days. Animals were observed twice per day for mortality, once per day for clinical signs, and body weight and food consumption were measured weekly. Ophthalmoscopic examinations were performed before the start of treatment and at its conclusion. Haematology and clinical chemistry parameters were determined at termination, after which all animals were given a macroscopic examination and selected organs were weighed. All tissues from rats in the control group and groups at 500 and 900 ppm were subjected to microscopic examination, along with lungs, liver, and kidneys from all groups. The mean test substance consumption for rats at 10, 50, 150, 250, 500, and 900 ppm was, respectively, 0.7, 3.3,

10, 17, 34 and 68 mg/kg bw per day for males and 0.8, 4, 12, 20, 38 and 80 mg/kg bw per day for females.

Mortality was limited to the group at 900 ppm, with all females and seven males dying at this dose. Relative to controls, body-weight gains were reduced in males at 500 ppm (15%) and 900 ppm (63%). In females, body-weight gains were reduced by 15% at 150 ppm, 10% at 250 ppm and 21% at 500 ppm. Food consumption followed a similar pattern to body weight in males, with reductions of 11% and 22% at 500 ppm and 900 ppm respectively, with food consumption in females at 500 ppm reduced by 12%. Observed clinical signs were restricted to the group at 900 ppm and comprised abdominal staining, ataxia, chromorhinorrhea, decreased faeces, dehydration, splayed hindlimbs, unthriftiness, clonic convulsions, and hypersensitivity to touch and sound. There were no toxicologically or statistically significant haematological findings in females at any dose. In males at 900 ppm, there were reductions in leukocytes ($5.5 \times 10^6/mm^3$ compared with the control value of $8.7 \times 10^6/mm^3$) and erythrocytes ($6.8 \times 10^6/mm^3$ compared with $8.0 \times 10^6/mm^3$ in the controls).

A number of significant differences occurred in various biochemical parameters in both sexes. Total protein was increased in males at 250 and 500 ppm (7.2 g/dl compared with 6.7 g/dl in rats in the control group), but did not differ from controls at 900 ppm. In females, total protein was decreased at all doses greater than 10 ppm (6.5–6.7 g/dl vs 7.5 g/dl in controls). In general, biochemical differences were not dose-related, were not consistent across both sexes, were small in magnitude (generally less than 10%), and in many cases were statistically significant owing predominantly to tight clustering of individual values at some doses, and unlikely to be toxicologically significant. In males, blood urea nitrogen values were substantially increased at 900 ppm (27 mg/dl vs 18 mg/dl in controls) and this was probably a treatment-related effect. In females, a 16% reduction in blood glucose concentrations at 500 ppm was observed, but was not seen in males at 500 or 900 ppm. In males at 900 ppm, absolute weights of heart, kidney and liver were less than controls by 23%, 21% and 37% respectively, and the relative weights of brain, adrenals, testes and heart were increased by 55%, 75%, 46% and 23%, respectively. In females at 500 ppm, the relative weights of brain, heart and kidneys were increased by 10%, 8% and 8% respectively. The differences in organ weights relative to corresponding control values were largely attributable to reduced body-weight gain. No treatment-related changes in gross pathology or histomorphology were observed at necropsy.

The NOAEL was 250 ppm, equal to 17 mg/kg bw per day for males and 20 mg/kg bw per day for females, on the basis of decreased body-weight gain at 500 ppm (34/38 mg/kg bw per day for males/females) (McCarty, 1990).

(a) Dermal

In a 21-day repeat-dose dermal study, zeta-cypermethrin (purity, 86%; total cypermethrin isomers, 95.7%) was applied undiluted to the clipped dorsal surface of Charles River Sprague-Dawley CD rats (10 of each sex per group) at a dose of 0, 100, 500 or 1000 mg/kg bw per day, occluded, for 6 h on consecutive days. At the end of each exposure period, the test sites were wiped with methanol, then rinsed with tap water and dried. Animals were observed for mortality twice per day and detailed physical examinations were performed daily. Food consumption and body weight were assessed weekly, as was irritation at the application site. Ophthalmological examinations were performed on all animals before testing and at the end of the study. On the day after the final dose, all animals underwent FOB and motor activity testing before necropsy. Haematology and clinical chemistry parameters were evaluated, selected organs were weighed (including thymus, but not thyroid) and tissues taken for histopathology (including treated and untreated skin).

No treatment-related clinical signs were observed, and body-weight gain and food consumption were similar in control and treated groups. Hindlimb grip strength in males at 1000 mg/kg bw per day was less than other groups, but this was mainly due to sporadic atypically high values in the

control and other treated groups (Table 11). Motor activity was decreased relative to controls in males at 500 and 1000 mg/kg bw per day. However, the difference at 500 mg/kg bw per day was largely due to a particularly low score in one animal, and at 1000 mg/kg bw per day, only two out of 10 session totals were below the range for controls. One female at the highest dose showed moderately impaired gait, uncoordinated landing (righting reflex), uncoordinated movement/ataxia, walking on toes, and mobile abnormal posture. As there were no other behavioural abnormalities in treated males or females the Meeting considered that none of these findings was due to exposure to zeta-cypermethrin.

Table 11. Statistically significant FOB and motor activity findings in rats receiving zeta-cypermethrin as a dermal application

Parameter	Sex	Dose (mg/kg bw per day)			
		0	100	500	1000
Hindlimb grip strength	M	0.413	0.387	0.400	0.324[§]
	F	0.269	0.315	0.301	0.258
Motor activity	M	304	307	234[**]	270[*]
(number of breaks/beam)	F	339	290	315	314

From Freeman (1999c)

F, female; M, male.

[§]$p = 0.034$; [*]$p = 0.027$; [**]$p = 0.005$; Welch trend test.

No treatment-related effects were detected at the ophthalmoscopic examinations. In males at 1000 mg/kg bw per day, haemoglobin and MCH were decreased to a statistically significant extent relative to controls ($p \leq 0.3$, Welch trend test), but as changes were small (2–3%) and results for individual rats at 1000 mg/kg bw per day were all within the range for concurrent controls, they were not considered to be biologically significant. Local irritation (erythema, eschar, desquamation and/or ulceration) was observed at the application sites in some treated animals, mainly at the 2- and 3-week examinations, at ≥ 100 mg/kg bw per day in females and ≥ 500 mg/kg bw per day in males. Macroscopic and microscopic examination of these sites indicated that many of these reactions were associated with trauma, were focal in nature, and possibly the result of self-excoriation or clipping. However, there were no similar findings in control animals, so it is possible that these findings were due to the test material. There were no other treatment-related histopathological findings. Statistically significant changes in clinical chemistry were limited to the groups at 1000 mg/kg bw per day and comprised decreased AST activity in both sexes, increased ALT activity in males, and increased chloride concentrations but decreased sorbitol dehydrogenase (SDH), sodium and blood urea nitrogen values in females (Table 12). The decreases in AST and SDH are not considered to be adverse effects, and considering that the increase in ALT in males occurred in the absence of a change in SDH, this is not considered to be a toxicologically significant finding. As sodium concentrations in females lacked a dose–response relationship, chloride was only increased by 3%, and blood urea nitrogen values were within the normal range for rats of this strain, none of these was considered to have toxicological relevance.

Table 12. Statistically significant clinical chemistry findings in rats receiving zeta-cypermethrin as a dermal application

Parameter	Sex	Dose (mg/kg bw per day)			
		0	100	500	1000
AST (U/l)	M	118	111	99	98*
	F	102	99	85	84**
ALT (U/l)	M	50	59	51	61§
	F	47	49	45	48
SDH (U/l)	M	14	16	14	14
	F	16	11	11	12^
Na	M	144	144	143	143
	F	143	141	141	142ª
Cl	M	103	103	103	103
	F	97	98	98	100¤
BUN	M	15	17	14	14
	F	15	15	14	13¨

From Freeman (1999c)

BUN, blood urea nitrogen; SDH, sorbitol dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

*p = 0.015; **p = 0.001; §p = 0.045; ^p = 0.001; ªp = 0.018; ¤p = 0.045; ¨p = 0.029; Welch trend test

In females at 1000 mg/kg bw per day, kidney, adrenal and spleen weights were increased relative to controls to a statistically significant degree (Table 13). As the changes in kidney and liver weight lacked a dose–response, and the dose–response for relative spleen weights was flat across a 10-fold dose range, with no supporting microscopic findings, these were not considered to be adverse. The NOAEL was 1000 mg/kg bw per day, the maximum dose tested (Freeman, 1999c).

Table 13. Statistically significant organ weights relative to body weight (%) in rats receiving zeta-cypermethrin as a dermal application

Organ	Sex	Dose (mg/kg bw per day)			
		0	100	500	1000
Kidneys	M	0.795	0.808	0.766	0.813
	F	0.742	0.832	0.875	0.834*
Adrenals	M	0.017	0.017	0.016	0.016
	F	0.027	0.032	0.032	0.033**
Spleen	M	0.202	0.196	0.209	0.208
	F	0.215	0.235	0.246	0.248§

From Freeman (1999c)

F, female; M, male.

*p = 0.004; **p = 0.009; §p = 0.003; Welch trend test

5.3 Genotoxicity

The results of assays for genotoxicity with zeta-cypermethrin are summarized in Table 14.

Table 14. Results of studies of genotoxicity with zeta-cypermethrin

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation (Ames)[a]	*S. typhimurium*, TA98, TA100, TA1535, TA1537, TA1538	100–10000 µg/plate in DMSO	88.2	Negative −S9 Positive +S9[b]	Batt (1990)
Gene mutation[e]	Chinese hamster ovary cells H*gprt* locus	1–1000 µg/ml (solvent not specified)	88.2	Negative	Sigler & Harbell (1990)
Chromosome aberration[a]	Chinese hamster ovary cells	255–1020 µg/ml in DMSO	88.1	Negative	Murli (1990)
Unscheduled DNA synthesis	Rat primary hepatocytes	14–4500 µg/ml in DMSO[c]	88.2	Negative	Curren (1989)
In vivo					
Chromosome aberration[d]	Rat bone marrow	31.25, 62.5, 125 mg/kg bw, single gavage dose in corn oil; killed 6, 18 or 30 h after dosing	82.6 (*s*-isomer) 91.9 (total isomers)	Negative	Murli (1993)

DMSO, dimethylsulfoxide; S9, 9000 × *g* supernatant from livers of male Sprague-Dawley rats.

[a] Duplicate experiments, with and without metabolic activation. GLP and QA statements included.

[b] Zeta-cypermethrin produced a small but reproducible and dose-dependent increase in the frequency of revertants in *S. typhimurium* TA100 in the absence of metabolic activation in two separate assays (mean revertants per plate: 128, 123, 123, 123, 195, 260 at concentrations of 0, 100, 333, 1000, 3333 and 10 000 µg/plate respectively in the first study, and 130, 133, 180, 196, 203 and 261 at 0, 333, 1000, 3333, 5000 and 10 000 µg/plate respectively in the replicate study. The test was negative in the presence of S9 mix, and in the other strains tested.

[c] GLP and QA statements included. Small macroscopic droplets of the test material were observed in the culture medium at ≥ 150 µg/ml, and microscopically at all concentrations, even though the test material appeared completely soluble in the chosen solvent (DMSO).

[d] GLP and QA statements included.

[e] Positive controls included; QA but no GLP statements included.

5.4 Reproductive toxicity

(a) Multigeneration studies

Male and female Sprague-Dawley rats (Crl:CD(SD)BR) were given diets containing zeta-cypermethrin technical (purity, 89.6%) in the diet at concentrations of 0, 7.5, 25, 100, 375 or 750 ppm for two generations. The parental (F_0) generation (30 of each sex per dose) was pretreated for 12 weeks then paired for 21 days for mating, after which males were sacrificed. F_0 females that littered were sacrificed on day 28 of lactation. Females that failed to litter were sacrificed on the 25th day after mating. Where possible, at least one pup of each sex was selected from each litter to make up the 20 animals of each sex per group of the F_1 generation. Pups not selected for breeding the second generation were sacrificed and necropsied. Evidence of toxicity was assessed according to general physical condition, body-weight increases, food consumption, litter sizes and viability, fertility, duration of gestation, and gross pathology and histology at necropsy. At concentrations of 7.5, 25, 100, 375 and 750 ppm, average achieved doses in the F_0 in the period before mating were 0.4, 1.5, 6, 22 and 43 mg/kg bw per day, respectively, for males and 0.6, 1.9, 7, 28 and 53 mg/kg bw per day for females. Achieved doses were similar in females during lactation, and in the F_1 parental generation.

There were four deaths in the F_0, comprising one control male and three females (one at 100 ppm and two at 750 ppm), the female deaths occurred during days 18–28 of lactation, and were considered to be treatment-related at 750 ppm. In addition, four females were sacrificed in error on day 21 (two at 750 ppm and one each at 100 and 375 ppm). At 750 ppm, male body weight and food consumption

were reduced by 13% relative to controls, with food consumption reduced by 12% in 375 ppm males. Relative to the control group, maternal body-weight gain was reduced by 35% at 750 ppm, and marginally (10%) at 375 ppm during the period before mating. Body weight was not affected during gestation, but during lactation the group at 750 ppm lost weight for days 1–4 (−7.4 g vs + 4.4 g in the control group), days 10–14 (−12.1 g vs + 6.3 g in the control group), but weight gain was markedly increased in this group for days 21–28 of lactation (+ 13.6 g vs −4.4 g in the control group). In the group at 375 ppm, maternal body-weight gains were generally lower than those of the controls during lactation, although not to a statistically significant extent. Food consumption for F_0 females followed a similar pattern to body weight, being significantly reduced (17%) throughout the period before mating, marginally reduced (10%) during gestation, and significantly reduced (41%) during lactation. At 375 ppm, maternal food consumption during lactation was also significantly reduced (17%) relative to controls. At 750 ppm, a significant number of F_0 males had soft or liquid faeces, and in maternal animals emaciated appearance, ataxia, hypersensitivity to sound and clonic convulsions were observed during lactation, a period during which food consumption and achieved dose were the highest (0.9, 2.9, 12, 41 and 67 mg/kg bw per day at doses of 7.5, 25, 100, 375 and 750 ppm respectively). Treatment did not affect reproductive and developmental indices, and there was no evidence of teratogenicity or embryotoxicity in the F_1 pups. However, at 750 ppm, 12 out of 30 dams had total litter loss before postnatal day 28, compared with 0 of 30 in the control group. Of 331 pups born live at this dose, 150 died before day 28 (140 of 189 those remaining post-cull on day 4 post partem), compared to 2 of 285 in the control group (0 of 186 post-cull). Body weights were reduced by 10–14% in pups of the group at 375 ppm, and by more than 50% at 750 ppm at postnatal day 21 (21 g vs 47 g in the control group). OWing to the high mortality rate in pups of the group at 750 ppm, this group was discontinued at week 3 after weaning. No dose-related abnormalities were observed at necropsy.

After weaning, 27 of 30 of the male and 24 of 27 of the female F_1 pups from the group at 750 ppm that survived to day 1 after weaning died. Animals in this group were generally small, ataxic, and had whole body tremors. Some were hypersensitive to sound or very weak, and a few of the females had clonic convulsions. At necropsy, some of the 750 ppm animals had dried red substance around the mouth, urine stained abdominal fur, small testes (expected in small male rats), gaseous distention of the stomach, and evidence of gastric erosions. At 375 ppm, one rat of each sex displayed hypersensitivity to sound. A significantly increased incidence of alopecia was observed at 375 ppm in males (14 out of 40 vs 2 out of 40 in the control group) and females (9 out of 23 vs 0 out of 24 in the control group) during the period before mating. The significant reductions in body weights observed in F_1 pups at 375 and 750 ppm before weaning continued after weaning, with the body weights of males at 375 ppm remaining approximately 10% below that of controls. However, body-weight change was equivalent in all groups of males from day 29 after weaning onwards. In F_1 females, body-weight gain was largely unaffected by treatment, and although the weights of the group at 375 ppm remained consistently below those of the controls, after weaning the difference rapidly became marginal (5–10%). Organ weights (absolute and relative to body weight) in the F_1 were largely unaffected by treatment. Relative brain weights were increased at 375 ppm by about 10% in both sexes, considered an effect of reduced body weight. Reproductive and developmental indices were unaffected in the F_1 parents and F_2 pups. Pup body weights in the F2 at birth and through to day 7 were not affected by treatment, but were significantly reduced at 375 ppm at postnatal days 14 and 21. There were no gross or microscopic findings in the F_1 adults or F_2 pups that were attributable to treatment. Overall, there were no effects on reproductive performance at the doses tested (750 ppm maximum in the first generation and 375 ppm in the second).

The NOAEL for pup development was 100 ppm, equal to 6 mg/kg bw per day, on the basis of decreased pup weight gain and clinical signs at 375 ppm, equal to 22 mg/kg bw per day. The NOAEL for adults was 100 ppm, equal to 6 mg/kg bw per day, on the basis of decreased body-weight gain and reduced food consumption during the periods before mating and lactation in the F_0, and clinical signs in some F_1 adults at 375 ppm, equal to 22 mg/kg bw per day (Hoberman, 1991).

(b) Developmental toxicity

Rats

Groups of 25 female Sprague Dawley rats (Crl:CD(SD)BR) were given zeta-cypermethrin (purity, 89.6%) at a dose of 0, 5, 12.5, 25 or 35 mg/kg bw per day in corn oil by gavage from day 6 to day 15 post coitum. Clinical observations and measurement of body weight and food consumption were performed daily. Pathological investigations were conducted at the end of the study period (day 20 of presumed gestation). The ovaries and uteri of each animal were removed and examined for the number of corpora lutea, and the number and position of implantations, including the numbers of live fetuses, early and late resorptions, and dead fetuses. The sex and weight of each fetus was determined, and all fetuses were examined for external malformations. Approximately half the fetuses were examined for skeletal malformations and the remainder for visceral abnormalities. No animals died before sacrifice. Clinical observations related to toxicity were limited to the groups at 25 and 35 mg/kg bw per day and primarily comprised ataxia, hypersensitivity, urine-stained abdominal fur, emaciated appearance and soft faeces. Tremors and convulsions were seen only in one animal at 35 mg/kg bw per day. Maternal necropsy findings were minimal and not dose-related, with a single animal at 35 mg/kg bw per day having white cloudy fluid in the thorax and pericardium adhering to the diaphragm, and two animals in the group at 5 mg/kg bw per day having a mammary mass or a lesion on the back and base of the tail. Relative to the control group, maternal body-weight gains were significantly reduced by 23% and 51% at 25 and 35 mg/kg bw per day, respectively, during the dosing period, with an associated reduction in food consumption of 10% and 24%, respectively. Treatment did not affect the average number of resorptions or fetuses, nor did it affect the number of dams with viable fetuses. No treatment-dependent differences in average fetal body weights, sex ratios, percentage dead or resorbed conceptuses were observed. The numbers of fetuses with alterations (gross external, visceral and skeletal combined) were increased slightly at higher doses (7 out of 390, 9 out of 377, 8 out of 321, 11 out of 384, and 12 out of 347 in increasing order of dose, not statistically significant), but were not considered to be an effect of treatment, as all abnormalities considered individually were reported as common in this strain of rat, occurred at fetal or litter incidences that were not dose-dependent and/or statistically significantly increased relative to controls, and were within the ranges for their respective historical controls.

The NOAEL for maternal toxicity was 12.5 mg/kg bw per day on the basis of clinical signs of toxicity and reduced weight gain at 25 mg/kg bw per day and higher. As there was no evidence of treatment-related effects on fetal developmental or survival in this study, the NOAEL for developmental toxicity was 35 mg/kg bw per day, the highest dose tested (Hoberman, 1990).

5.5 Special studies

(a) Neurotoxicity

In a study of acute neurotoxicity, groups of 10 male and 10 female Long Evans rats (Charles River Laboratories) were given zeta-cypermethrin (FMC 56701; purity, 84.4%) undiluted as a single dose at 0 (tap water only), 10, 50 or 250 mg/kg bw by gavage on day 0. The animals were observed twice per day for mortality, a detailed physical exam was performed daily, and body weight was measured weekly. FOB and testing for motor activity were conducted for two of each sex per group before treatment and on days 0, 7 and 14. After testing on day 14, five of each sex in the control group and in the group at 250 mg/kg bw were anaesthetized, perfused in situ with fixative, and given a gross necropsy. Tissue samples from the nervous system (brain, spinal cord, sciatic, tibial and sural nerves, Gasserian ganglion, cervical and lumbar dorsal root fibres and ganglia, cervical and lumbar ventral root fibres), and gastrocnemius muscle were taken for histopathological examination. One female at 250 mg/kg bw died on day 0 after displaying treatment-related clinical signs. At 250 mg/kg bw,

splayed hindlimbs, staggered gait, convulsions, ataxia, loss of muscle control, oral discharge, abdominal staining, abdominal gripping, lacrimation, vocalization and decreased locomotion were observed, with splayed hind limbs, staggered gait and tremors also apparent in males at 50 mg/kg bw. All clinical signs resolved within 48 h of dosing. Additional findings at 250 mg/kg bw in the FOB on the day of dosing were impaired or absence of righting reflex, increased latency to tail flick, and tenseness/rigidity or limpness/lack of resistance during handling. There were no treatment-related findings in motor activity testing. Body weight was not affected by treatment, nor were there any treatment-related gross lesions or neuropathological findings at microscopic examination.

The NOAEL for acute neurotoxicity was 10 mg/kg bw on the basis of clinical signs and FOB findings at 50 mg/kg bw (Watt, 1998).

In a 90-day study of neurotoxicity, groups of 10 male and 10 female Charles River Long Evans rats were given diets containing zeta-cypermethrin (FMC 56701; purity, 86.0%) at a concentration of 0, 75, 400 or 750 ppm. The animals were observed twice per day for mortality and daily for clinical signs, and body weights and food consumption were measured weekly. Motor activity and FOB assessments were conducted on all rats before treatment and after 4, 8 and 13 weeks of treatment. At study termination, five rats of each sex per group were anaesthetized and perfused with fixative in situ. Tissue samples from the nervous system (brain, spinal cord, sciatic, tibial and sural nerves, Gasserian ganglion, cervical and lumbar dorsal root fibres and ganglia, cervical and lumbar ventral root fibres), and gastrocnemius muscle were taken for histopathological examination. The mean daily intake of zeta-cypermethrin at 75, 400 and 750 ppm was 5.0/5.9, 26.3/31.5 and 47.2/55.6 for males/females, respectively. There were no treatment-related deaths or clinical signs, nor were there any gross pathology or neuropathology findings that could be attributed to treatment. Overall body-weight gain was reduced by 28% and 31% in males and females respectively at 750 ppm, and by 15% in males at 400 ppm. At 750 ppm, this was associated with a reduction in food consumption of 13% and 11% respectively in males and females. In males at 400 and 750 ppm, relative to control animals, landing foot-splay was reduced in week 13, and motor activity was decreased at weeks 8 and 13. Tail-flick latency was decreased in these groups at week 8 only, but as this was not present at the later time-point, was not attributed to treatment.

The NOAELs for neurotoxicity were 75 ppm for male rats (5.0 mg/kg bw per day), on the basis of increased landing foot-splay and decreased motor activity at 400 ppm (26 mg/kg bw per day), and 750 ppm (56 mg/kg bw per day) for female rats, the highest dose tested. The NOAELs for systemic toxicity were 75 ppm for male rats (5.0 mg/kg bw per day) and 400 ppm (31.5 mg/kg bw per day) for female rats, on the basis of decreased body-weight gain and food consumption at 400 ppm (47 mg/kg bw per day) and 750 ppm (56 mg/kg bw per day), respectively (Freeman, 1999d).

6. Observations in humans exposed to cypermethrins

6.1 Medical surveillance of manufacturing plant personnel

Zeta-cypermethrin technical, along with other synthetic pyrethroids, has been produced on a batch basis at a manufacturing plant in Baltimore, Maryland, USA, since 1993. It was estimated that employees worked with zeta-cypermethrin about 15% of their time at work. Twenty-five employees had worked at the facility for 10 years. All employees participated in the Medical Surveillance Program at the plant, which included pre-employment baseline and annual measurements, comprising spirometry, audiometric testing, electrocardiogram (ECG), chest X-ray, vision screening, hands-on examination by a physician, blood chemistry, haematology and complete urine analysis. There were no unexplained or significant changes from baseline, or variations from reference ranges for employees working with synthetic pyrethroids. The only health effect associated with zeta-cypermethrin was

mild and temporary paraesthesia resulting from skin contact. A review of medical records of affected employees still employed at the manufacturing plant did not identify any subsequent health problems (Sheffield & Kerschner, 2003).

Similar findings were reported for a manufacturing plant in Jacksonville, Florida, at which a product containing zeta-cypermethrin has been formulated and packaged, and a related product also packaged, since 1993. In this case, employees, 100 of whom had been employed at the plant for a significant period of time during the 10-year period covered by this report, were working with products containing zeta-cypermethrin about 20% of their time at work (Chlada, 2003).

6.2 Exposure of the general population

A group of nine workers were exposed to cypermethrin after accidental contamination of an air-conditioning duct during slab injection with a diluted termiticide product containing 0.25% cypermethrin. The product also contained aromatic petroleum solvents (including xylene), plus trimethylbenzene and paraffinic oils. The employees entered the building on several occasions 2 days after it was treated, and again at 13 days after treatment, at which time the airborne concentrations of cypermethrin were below the limit of detection (0.02 ppm). Six weeks after treatment, though air-sampling tests were negative, wipe tests demonstrated cypermethrin in the carpet, with the highest concentration between two applicator holes. Symptoms common to five employees were shortness of breath, cough, congestion, burning of the eyes, and itching of the skin at the time of exposure. Dizziness, headaches, nausea and vertigo were also reported. The symptoms occurred each time the workers entered the building, worsened when the air conditioning was activated, and appeared to be exaggerated in smokers, though one non-smoker developed significant pulmonary dysfunction. The five workers reporting symptoms also experienced extreme anxiety. A further three workers reported no symptoms, and the remaining worker was unavailable for examination. The paper stressed the importance of full decontamination before re-entry and rapid referral of exposed individuals to a specialist in toxicological exposures (Lessenger, 1992).

A prospective epidemiological study was conducted from 1996 to 1999 to determine the systemic exposure of 40 male and 21 female volunteers exposed to pyrethroids after the indoor application of pyrethroid-containing products by professional pest control operators. Forty subjects were exposed to cyfluthrin, nine to permethrin, seven to cypermethrin and five to deltamethrin. Persons with various chronic medical conditions or a history of use of a pest-control operator in the 6 months before the study were excluded. They were admitted to treated premises at 8 h after treatment, following a 4-h period during which the premises were ventilated. Biological monitoring was performed before pesticide treatment and at 1 day, 4–6 months and 10–12 months after treatment. The study included general medical and neurological examination of the volunteers, a question-based interview, and blood and urine sampling to determine general clinical and immunological parameters, as well as biological monitoring. The concentration of the appropriate pyrethroid was determined in the plasma, and metabolites were determined in the urine. The ratio of *trans*-DCCA (3-(2,2-dichlorovinyl)-2,2-dimethylcyclo-propane carboxylic acid) to *cis*-DCCA was calculated to investigate whether the major route of uptake was via the dermal (ratio ≤ 1) or the inhalation/oral routes (ratio > 1). In all cases, the plasma concentration of pyrethroid was below the limit of detection (< 5 µg/l). A significant increase in the concentration of pyrethroid metabolites was found in the urine for days 1–3 after application of pyrethroid, but thereafter did not exceed published background levels. The route of exposure was ascribed to the inhalation/oral route for thirteen subjects and to the dermal route for five subjects (Leng et al., 2003).

During 1989–2003, 60 poisoning incidents with pesticides containing cypermethrin were recorded in the mandatory German reporting scheme, accessible through the INTOX database. Of these, 29

related directly to symptoms in humans, comprising two unsuccessful suicide attempts, four accidental child exposures without any serious effects, and 23 cases of either household exposure or professional use. All recorded cases included mild symptoms of vertigo, nystagmus (eye), drowsiness, emesis, hypertonia, nausea, headache, lassitude, anorexia, redness in the face, pyrexia, swelling of the lymph nodes, slight conjunctivitis, cauterization of the eyes, slight effects on the skin and ears and laboured breathing. There were no deaths, and no incidents were considered to be serious (Ullrich, 2003).

6.3 Occupational exposure

Urine obtained from operators spraying cypermethrin in experimental trials was analysed for the presence of the chlorinated cyclopropanecarboxylic acid metabolite. Using standard gas–liquid chromatography, the carboxylic acid metabolite was observed in urine of exposed workers at concentrations up to 0.4 µg/ml (the limit of detection was estimated to be 0.05 µg/ml) (Annex 1, reference *33*; Baldwin, 1978).

Studies were carried out in the Ivory Coast, Africa where operators sprayed with a hand-held ultra-low volume (ULV) apparatus either cypermethrin or a control UW formulation. The cypermethrin sprayers were found to have residues on the exposed parts of their bodies. Medical and electrophysiological examinations showed no adverse effects from the exposure. The rate of dermal exposure of the operators during spraying ranged from 1.5 to 46.1 mg/h. There was a reasonable relationship between the total cypermethrin deposited dermally and the excretion in urine. The concentrations of the cyclopropanecarboxylic acid metabolite in the urine at 24 h were between < 0.05 and 0.32 mg. This, together with the finding of 0.6 mg of this metabolite in 72 h urine from one man led to the estimation that approximately 3% of the total dermal dose was absorbed and rapidly excreted by the operators (Annex 1, reference *33*; Prinsen & van Sittert, 1978).

A study was carried out in humans at the Institut de Recherches du Coton et des Textiles Exotiques (IRCT), Bouake, Ivory Coast, after hand-held ULV application to cotton of the cypermethrin (‘Ripcord’) during one season of spraying. The operators were seven indigenous Africans. Six of them had no previous pesticide application history; one had very limited experience. They sprayed the formulation EF 4922 (25 g Ripcord/l in a 50/50 (v/v) hexylene glycol and Shellsol AB mixture) on an area of 1 ha (10 000 m^2) at the rate of 2 l/10 000 m^2 in six spray sessions at fortnightly intervals under the supervision of IRCT staff. In a seventh spray session, the same amount was sprayed under the supervision of the Exposure Monitoring Group. The operators wore their normal clothes and no special protective devices were used.

Exposure to Ripcord was monitored by collecting the total amount of urine passed during the first 24 h after each spray session and the determination of the excretion levels of the urinary metabolite *trans* WL 44776 3-(2,2-dichlorovinyl-2,2-dimethyl-cyclopropane-1-carboxylic acid). In many samples the excretion was below the limit of detection, indicating a low level of absorption. Skin contamination and inhalational exposure were monitored only in the seventh spray session. Skin and inhalational measurements did not correlate well with the urinary excretion. Inhalational exposure was about 1% of skin exposure. Skin exposure, as measured with aluminium foil, reflected the working procedures. It was concluded that exposures were low, in particular when the spraying recommendations were observed. The absorption was calculated to be substantially less than the equivalent of an oral dose of 1 mg of Ripcord per spray session. General medical and extensive clinical and neurological examinations, blood biochemistry and peripheral nerve function tests (including the trigeminal nerve) did not show abnormalities either before or after the series of six spray sessions nor after the seventh spray session. In some electroneurophysiological tests (motor conduction velocity, slow fibre conduction velocity and cornea reflex) a statistically significant change within the normal

range appeared to exist for the group of sprayers between the before and after exposure measurements. There was no evidence that these changes were compound-related; they probably reflected seasonal variations. The general conclusion is that under the conditions of this study the application of Ripcord caused no detectable adverse health effects (Annex 1, reference *33*; Prinsen & Van Sittert, 1979).

Transient facial sensory symptoms after exposure to synthetic pyrethroids (e.g. cypermethrin, permethrin, fenvalerate and fenpropathin) in some workers have been reported. Among 23 workers exposed to synthetic pyrethroids, 19 had experienced one or more episodes of abnormal facial sensation that developed between 30 min and 3 h after exposure and persisted for 30 min to 8 h. There were no abnormal neurological signs and electrophysiological studies were normal in the arms and legs. It was concluded that the symptoms are most likely to be due to transient lowering of the threshold of sensory nerve fibres or sensory nerve endings after exposure of the facial skin to pyrethroids, similar to the phenomena that have been described in Wouters & van den Bercken (1978), after exposure of animal nerves to pyrethroids (Annex 1, reference *37*; Le Quesne et al., 1981).

Skin sensations experienced by those handling cypermethrin or other pyrethroids are believed to arise by repetitive firing of sensory nerve terminals in the skin. It is said to be a strictly local effect, which may occur as soon as the concentration of pyrethroid on or in the skin reaches a certain level and is not considered as a sign of general intoxication (provided the pyrethroid does not reach the blood in any significant concentration). A possibility exists, however, that repeated occurrence of intense repetitive firing can perhaps eventually lead to dysfunction of sensory nerve terminals and sense organs and finally to degeneration of sensory nerve fibres (Annex 1, reference *33*; van den Bercken, 1980).

A monitoring study was undertaken to determine the dermal exposure of two pilots and two mixer-loaders, and the systemic absorption by five mixer-loaders during operations for the aerial application to cotton of a ULV formulation of cypermethrin in oil. Cypermethrin absorption was measured in three mixer-loaders by assessing the concentrations of the cypermethrin metabolites 3-(2,2-dichloroethenyl)-2-2-dimethylcyclopropanecarboxylic acid (DCEA, *cis* and *trans*), 3-phenoxybenzoic acid (3PBA) and 4′-hydroxy-3-phenoxybenzoic acid (4OHPBA) on the day of application and for 6 days thereafter. The formulation used (Cymbush 3E) contained 360 g/kg cypermethrin which was diluted in fully refined soya bean oil to a spray concentration of 2.9% w/v cypermethrin. Each replicate application used 15 l of Cymbush. All mixer-loaders wore protective equipment. Dermal exposure to cypermethrin was 0.67 mg/8 h for pilots, and 2.4 mg/8 h for mixer-loaders. For pilots, most exposure was to the hands, while exposure was more uniform for mixer-loaders, principally involving the arms, trunk and hands, reflecting a high degree of protection afforded by gloves. The metabolites 4OHPBA and 3PBA were detected in the urine of all three subjects, and *trans*-DCEA (but not *cis*-DCEA) was detected in two subjects. The most persistent urinary metabolite was 4OHPBA, which was not detected on day 4 in two subjects, and was just detectable in the third subject on days 5 and 6. It was concluded that as the major metabolites in the urine were 3PBA and 4OHPBA, metabolism and excretion in humans resembled that in rats more closely than that in mice. Calculations of cypermethrin excreted indicated that systemic absorption (46–78 µg of cypermethrin) represented a small proportion of actual dermal exposure (approximately 0.63–3.6 mg of cypermethrin) (Chester et al., 1987).

A review of 573 cases of acute pyrethroid poisoning reported in the Chinese medical literature during 1983–1988 showed that 45 cases were involved cypermethrin. Of these, six were due to occupational exposure and 39 were accidental. In most cases of occupational pyrethroid poisoning, effects were reversible, so the prognosis was generally good. Details specific to the cypermethrin cases were not provided. After occupational exposure, symptoms of burning or itching sensations

of the face which usually developed at 4–6 h after exposure were consistent with the paraesthesia reported elsewhere for cypermethrin, and were transient in nature (He et al., 1989).

An epidemiological survey of the prevalence of pyrethroid poisoning in cotton growers in Gaocheng County, China, was conducted in 1987/1988. A total of 3113 pyrethroid sprayers (2230 men, 883 women) were interviewed after spraying, and, and followed up over a 72-h period. Deltamethrin and fenvalerate were the main pyrethroids used, with only 12 subjects exposed to cypermethrin. About 25% of subjects were exposed to a pyrethroid/organophosphate mixture. A total of 834 subjects were clinically affected, reporting mainly abnormal facial sensations (burning and tingling) within 4 h after spraying commenced, and resolving within 24 h. Systemic symptoms of dizziness, headache, fatigue, nausea, and loss of appetite were also seen, but were described as minimal.

Ten subjects who developed significant systemic symptoms and showed listlessness or muscular fasciculation were diagnosed as having mild acute pyrethroid poisoning; all recovered in 3–4 days. A comparison of measured concentrations of pyrethroid in the breathing zone, on detection pads on the clothes/skin and in urine samples indicated that the predominant route of exposure was dermal. Causal factors for pyrethroid poisoning that emerged from the survey were lack of awareness of the toxicity of pyrethroids, unsatisfactory personal protection and unsafe work practices (Chen et al., 1991).

Comments

Biochemical aspects

The fate of orally administered cypermethrin was studied in mice, rats, dogs, and humans and alpha-cypermethrin was investigated in rats and humans. When administered orally to rats, cypermethrin and alpha-cypermethrin were partially absorbed, distributed widely in the tissues, and excreted rapidly. After a low single oral dose (2 mg/kg bw) of ^{14}C-labelled cypermethrin or alpha-cypermethrin, approximately 50–75% of the radioactivity was excreted in the urine, with little in the expired air, and the remainder in the faeces. As most of the radiolabelled material in the faeces comprised the unmetabolized parent molecule, the role of biliary excretion appears to be minor, although this was not measured directly, and the amount in the urine represents approximately the amount absorbed. Maximum concentrations in the blood were reached at 3–4 h after dosing at 2 mg/kg bw.

In rats given a single oral dose of ^{14}C-labelled cypermethrin or alpha-cypermethrin at 2 mg/kg bw, the highest tissue concentrations of radioactivity were found in the fat (< 1% of the administered dose), followed by the skin. In rats and mice, radioactivity in the fat was identified as unchanged cypermethrin, present mainly as the *cis*-isomer. Elimination of radiolabel from most tissues was rapid, but the elimination half-life of cypermethrin and alpha-cypermethrin in rodent adipose tissue and skin was prolonged (10–40 days). Repeat-dose studies in rats confirmed that cypermethrin accumulates in fat and skin, reaching a plateau after dosing for 4 weeks at 2 mg/kg bw per day. Concomitant increases in radioactivity also occurred in the plasma, liver and kidney, but concentrations were an order of magnitude lower than in fat.

In laboratory animals, cypermethrin was readily hydrolysed at the ester bond, followed by hydroxylation and conjugation of the cyclopropyl and phenoxybenzyl moieties of the molecule. Urinary metabolites consistent with a similar metabolic pathway in humans were recovered from orally dosed volunteers. The animal data indicated that there is little isomeric interconversion during metabolism of cypermethrin or alpha-cypermethrin.

Toxicological data

Cypermethrin has low to moderate acute oral toxicity in rats (LD_{50}, 163 to > 3000 mg/kg bw). This variability was only partly explicable by the vehicle used. The acute oral LD_{50} of *cis*-cypermethrin in rats was 160–300 mg/kg bw, indicating that it is considerably more toxic than *trans*-cypermethrin,

for which the LD_{50} was > 2000 mg/kg bw under the same conditions. From these results, it would be predicted that alpha-cypermethrin is approximately twice as acutely toxic as cypermethrin. A wide range of acute oral LD_{50} values in rats was also reported for alpha-cypermethrin (LD_{50}, 64 to > 5000 mg/kg bw). Similar studies with zeta-cypermethrin gave fairly consistent results (LD_{50}, 86–367 mg/kg bw). The dermal toxicity of cypermethrin and alpha-cypermethrin was low in rats (LD_{50}, > 1600 mg/kg bw and > 2000 mg/kg bw per day, respectively), as was the dermal toxicity of zeta-cypermethrin in rabbits (LD_{50}, > 2000 mg/kg bw), and inhalation toxicity was moderate for cypermethrin (LC_{50}, 1.260 mg/l) and alpha-cypermethrin (LC_{50}, 1.590 mg/l). Overall, the three isomeric mixtures displayed qualitatively similar profiles for acute toxicity in rats.

In rabbits, cypermethrin, alpha-cypermethrin and zeta-cypermethrin were slight eye irritants and slight skin irritants. Cypermethrin showed potential for skin sensitization in the maximization test in guinea-pigs, but was not a sensitizer according to the Buehler method, while alpha-cypermethrin was not a sensitizer in the maximization test, but zeta-cypermethrin was a skin sensitizer in the Buehler test. Cypermethrins also produce local paraesthesia (a tingling or burning sensation of the skin not associated with tissue damage) as an acute action that is distinct from irritancy.

Cypermethrin, alpha-cypermethrin and zeta-cypermethrin cause neurotoxicity in mammals and insects by causing a long-lasting prolongation of the normally transient increase in sodium permeability of nerve membrane channels during excitation. Salivation, and tremors that progress to clonic-tonic convulsions (choreoathetosis and salivation syndrome), along with gait abnormalities and ataxia are induced in rodents at high doses (> 100 mg/kg bw) but in dogs at lower doses (> 25 mg/kg bw), as seen in studies of acute toxicity and short-term studies of toxicity.

The main toxicological findings in repeat-dose studies in rodents were reduced weight gain, reduced food consumption, and at higher doses, signs of neurotoxicity (convulsions, tremors, hypersensitivity to touch and sound). Reduced weight gain and food consumption in rodents was observed with cypermethrin at dietary concentrations of 1000 ppm, equivalent to 50 mg/kg bw per day, and above. For alpha-cypermethrin these effects occurred at 100 ppm, equal to 11 mg/kg bw per day, in mice, while for zeta-cypermethrin the same effects were observed at 400 ppm, equal to 26 mg/kg bw per day, in rats. Dogs appeared to be the most sensitive species, with clinical signs of neurotoxicity being observed in the absence of body-weight loss at dietary concentrations of 600 ppm, equivalent to 15 mg/kg bw per day, and 120 ppm, equivalent to 3 mg/kg bw per day, for cypermethrin and alpha-cypermethrin respectively. Dogs dosed with alpha-cypermethrin for 3 months showed the usual clinical signs of pyrethroid toxicity, namely body tremors and variable incidences of head nodding, lip-licking, subduedness, ataxia, and agitation. The NOAEL for clinical signs in the 3-month study was 90 ppm, equivalent to 2.2 mg/kg bw per day. However, dogs dosed for 12 months showed no systemic toxicity. There was, however, abdominal skin reddening, skin reddening of the tail, including ulceration and necrosis of the tail in one male. The NOAEL for this effect was 60 ppm, equivalent to 1.5 mg/kg bw per day. There were no apparent methodological reasons for the disparity in clinical signs observed in the 3-month study in dogs, but not in the 12-month study in dogs. Similarly, the local skin irritation effects, possibly secondary to paraesthesia, observed after 3 weeks at 120 ppm, equivalent to 3 mg/kg bw per day, in the 12-month study were not found at higher doses (270 ppm, equivalent to 6.7 mg/kg bw per day) in the 3-month study. It was not possible to discount the possibility that this may have been caused by accidental contact with food containing alpha-cypermethrin. For zeta-cypermethrin, there were no studies in dogs. However, repeat-dose studies of neurotoxicity involving functional observational battery tests in rats given diets containing zeta-cypermethrin indicated reduced landing foot-splay and motor activity at 400 ppm, equal to 26 mg/kg bw per day.

There was no evidence of carcinogenicity with cypermethrin at dietary concentrations of up to 1600 ppm, equivalent to 240 mg/kg bw per day, in mice and at up to 1500 ppm, equivalent to 75 mg/kg bw per day, in rats. This was also the case in mice given diets containing alpha-cypermethrin at concentrations of up to 300 ppm, equal to 35 mg/kg bw per day, the highest dose tested.

Cypermethrin, alpha-cypermethrin and zeta-cypermethrin gave negative results in an adequate battery of studies of genotoxicity in vitro and in vivo.

In the absence of any carcinogenic potential in rodents and the lack of genotoxic potential in vitro and in vivo, the Meeting concluded that the cypermethrins are unlikely to pose a carcinogenic risk to humans.

In a three-generation study of reproductive toxicity in rats, adults receiving cypermethrin at a dietary concentration of 150 ppm, equal to 11 mg/kg bw per day, showed reduced body-weight gain and food consumption, and pups had lower body-weight gain during lactation at the higher dose of 750 ppm, equal to 56 mg/kg bw per day. Consistent with this, adult rats at 500 ppm, equivalent to 38 mg/kg bw per day, in a two-generation study of reproductive toxicity, also showed reduced body-weight gain and food consumption, but in this case litter size and litter weight were decreased at the same dose. In a two-generation study of reproductive toxicity with zeta-cypermethrin, decreased maternal body-weight gain and food consumption occurred at 375 ppm, equal to 22 mg/kg bw per day, along with decreased pup body weight. In contrast to the studies of reproductive toxicity with cypermethrin, clinical signs were observed in the dams and pups treated with zeta-cypermethrin at 22 mg/kg bw per day and higher, although similar NOAELs were obtained (6 mg/kg bw per day). No effects on reproductive performance were observed with either cypermethrin or zeta-cypermethrin.

In studies of developmental toxicity with cypermethrin and alpha-cypermethrin in rats and rabbits, and with zeta-cypermethrin in rats, teratogenicity was not observed. The only developmental effect noted in any of these studies was a slight but statistically significant reduction in fetal weight in rats treated with alpha-cypermethrin when clinical signs of neurotoxicity, and decreased body-weight gain and food consumption were seen in the dams. The NOAEL for these effects was 9 mg/kg bw per day. There were no developmental effects in rabbits given alpha-cypermethrin at up to 30 mg/kg bw per day or cypermethrin at 700 mg/kg bw per day, the highest doses tested, but alpha-cypermethrin was relatively more maternally toxic than cypermethrin in rabbits, causing a decrease in body-weight gain at 30 mg/kg bw per day, while the dose of cypermethrin at which similar effects were seen was 700 mg/kg bw per day.

Studies of acute neurotoxicity in rats were performed with cypermethrin, alpha-cypermethrin and zeta-cypermethrin. With cypermethrin, reduced activity and gait abnormalities were observed at a dose of 20 mg/kg bw; the NOAEL was 4 mg/kg bw. At doses of 60 mg/kg bw and above, salivation, choreoathetosis, altered righting reflex, splayed limbs and flattened posture were observed; urination, landing foot-splay and click response were increased; and arousal, grip strengths, touch response and tail-pinch response were decreased. Alpha-cypermethrin induced death, clinical signs, gait abnormalities, abnormal reactivity in the FOB, and slight to very slight degeneration of sciatic nerve fibres at doses of 20 mg/kg bw and above, with males being more severely affected than females. The NOAEL was 4 mg/kg bw. With zeta-cypermethrin at a dose of 50 mg/kg bw, clinical signs were observed, with additional findings of FOB abnormalities and one female death at 250 mg/kg bw. The NOAEL was 10 mg/kg bw.

Overall, the limited database for zeta-cypermethrin indicated that its toxicity profile was similar to that for cypermethrin and alpha-cypermethrin.

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

Toxicological evaluation

The Meeting acknowledged that since racemic cypermethrin already includes a substantial proportion of alpha- and zeta-cypermethrin, and that all three cypermethrins are qualitatively similar in their toxicity and metabolism, an ADI established for alpha-cypermethrin could apply for all three

substances. Since conventional testing of cypermethrin residues in treated commodities is unable to distinguish between the isomers, a group ADI is appropriate.

The Meeting established a group ADI of 0–0.02 mg/kg bw per day based on a NOAEL of 2.2 mg/kg bw per day for severe clinical signs of neurotoxicity in a 3-month dietary study in dogs treated with alpha-cypermethrin, and using a 100-fold safety factor. This NOAEL is supported by a similar NOAEL of 1.5 mg/kg bw per day for abdominal skin reddening and alopecia in a 12-month dietary study in dogs.

The Meeting established a group ARfD of 0.04 mg/kg bw based on the NOAEL of 4 mg/kg bw, and using a 100-fold safety factor. The NOAEL observed in a study of acute neurotoxicity was on the basis of death, clinical signs, changes in FOB tests and degenerative changes to the sciatic nerve at higher doses. Although the database indicated that dogs were more sensitive than rats to neurotoxic effects, the delayed onset of clinical signs (2 days) after dosing at 6.75 mg/kg bw per day in the 3-month study in dogs suggests that the NOAEL in the study of acute toxicity in rats would also be adequate for the most sensitive species.

Levels relevant to risk assessment

(a) Cypermethrin

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	400 ppm, equivalent to 60 mg/kg bw per day	1600 ppm, equivalent to 240 mg/kg bw per day
		Carcinogenicity	1600 ppm, equivalent to 240 mg/kg bw per day[d]	—
Rat	Three-month studies of toxicity[a,b]	Toxicity	400 ppm, equivalent to 40 mg/kg bw per day	1500 ppm, equal to 116 mg/kg bw per day
	Two-year studies of toxicity and carcinogenicity[a,b]	Toxicity	150 ppm, equivalent to 7.5 mg/kg bw per day	1000 ppm, equivalent to 50 mg/kg bw per day
		Carcinogenicity	1500 ppm, equivalent to 75 mg/kg bw per day[d]	—
	Multigeneration reproductive toxicity[a,b]	Parental toxicity	50 ppm, equal to 3.8 mg/kg bw per day	150 ppm, equal to 11 mg/kg bw per day
		Offspring toxicity	100 ppm, equivalent to 7.5 mg/kg bw per day	500 ppm, equivalent to 38 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	17.5 mg/kg bw per day	35 mg/kg bw per day
		Embryo/fetotoxicity	70 mg/kg bw per day[d]	—
	Acute neurotoxicity[c]	Neurotoxicity	4 mg/kg bw	20 mg/kg bw
Rabbit	Developmental toxicity[b,c]	Maternal toxicity	450 mg/kg bw per day	700 mg/kg bw per day
		Embryo/fetotoxicity	700 mg/kg bw per day[d]	—
Dog	Three-month studies of toxicity[a,b]	Toxicity	500 ppm, equivalent to 12.5 mg/kg bw per day	800 ppm, equal to 25 mg/kg bw per day
	One-year study of toxicity[a]	Toxicity	200 ppm, equal to 5.7 mg/kg bw per day	600 ppm, equal to 18 mg/kg bw per day
	Two-year study of toxicity[a]	Toxicity	300 ppm, equivalent to 7.5 mg/kg bw per day	600 ppm, equivalent to 15 m g/kg bw per day

[a] Dietary administration.

[b] Two or more studies combined.

[c] Gavage administration.

[d] Highest dose tested.

(b) Alpha-Cypermethrin

Species	Study	Effect	NOAEL	LOAEL
Mouse	Three-month study of toxicity[a]	Toxicity	50 ppm, equal to 6.3 mg/kg bw per day	250 ppm, equal to 33 mg/kg bw per day
	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	30 ppm, equal to 3 mg/kg bw per day	100 ppm, equal to 10.6 mg/kg bw per day
		Carcinogenicity	300 ppm, equal to 35 mg/kg bw per day[d]	—
Rat	Three-month study of toxicity[a]	Toxicity	180 ppm, equivalent to 18 mg/kg bw per day	540 ppm, equivalent to 54 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	9 mg/kg bw per day	18 mg/kg bw per day
		Embryo/fetotoxicity	9 mg/kg bw per day	18 mg/kg bw per day
	Acute neurotoxicity[c]	Neurotoxicity	4 mg/kg bw	20 mg/kg bw
Rabbit	Developmental toxicity[c]	Maternal toxicity	15 mg/kg bw per day	30 mg/kg bw per day
		Embryo/fetotoxicity	30 mg/kg bw per day[d]	—
Dog	Three-month study of toxicity[a]	Toxicity	90 ppm, equivalent to 2.2 mg/kg bw per day	270 ppm, equivalent to 6.7 mg/kg bw per day
	One-year study of toxicity[a]	Toxicity	60 ppm, equivalent to 1.5 mg/kg bw per day	120 ppm, equivalent to 3 mg/kg bw per day

[a] Dietary administration.

[b] Two or more studies combined.

[c] Gavage administration.

[d] Highest dose tested.

(c) Zeta-Cypermethrin

Species	Study	Effect	NOAEL	LOAEL
Rat	Three-month study of toxicity[a]	Toxicity	250 ppm, equal to 17 mg/kg bw per day	500 ppm, equal to 34 mg/kg bw per day
	Multigeneration reproductive toxicity[a]	Parental and offspring toxicity	100 ppm, equal to 6 mg/kg bw per day	375 ppm, equal to 22 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	12.5 mg/kg bw per day	25 mg/kg bw per day
		Embryo/fetotoxicity	35 mg/kg bw per day[d]	—
	Acute neurotoxicity[c]	Neurotoxicity	10 mg/kg bw	50 mg/kg bw
	Three-month study of neurotoxicity[a]	Neurotoxicity	75 ppm, equal to 5 mg/kg bw per day	400 ppm, equal to 26 mg/kg bw per day

[a] Dietary administration.

[b] Two or more studies combined.

[c] Gavage administration.

[d] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.02 mg/kg bw

Estimate of acute reference dose

0.04 mg/kg bw

Information that would be useful for continued evaluation of the compound

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

Critical end-points relevant for setting guidance values for exposure to cypermethrins

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	T_{max}, approximately 3 h; approximately 50–70% absorbed
Dermal absorption	Approximately 1% in humans
Distribution	Throughout the body; highest levels in fat, present mainly as *cis*-isomers
Potential for accumulation	The elimination half-life in fat was 10–25 days after a single oral dose; radioactivity accumulated in fat and skin after repeated oral dosing
Rate and extent of excretion	Rapid; > 95% excreted in 48 h
Metabolism in animals	Extensive, no unchanged cypermethrin excreted in the urine
Toxicologically significant compounds (animals, plants, environment)	Parent
Acute toxicity (cypermethrin)	
Rat, LD_{50}, oral	200 to > 3000 mg/kg bw
Rat, LD_{50}, dermal	> 1600 mg/kg bw (xylene vehicle); > 4800 mg/kg bw undiluted
Rat, LC_{50}, inhalation	1.260 mg/l air
Guinea-pigs, skin sensitization (test method used)	Sensitizer (maximization); non-sensitizer (Buehler)
Acute toxicity (alpha-cypermethrin)	
Rat, LD_{50}, oral	64 to > 5000 mg/kg bw, depending on vehicle
Rat, LC_{50}, inhalation	1.590 mg/l air
Acute toxicity (zeta-cypermethrin)	
Rat, LD_{50}, oral	86–367 mg/kg bw (corn oil vehicle)
Short-term studies of toxicity	
Target/critical effect	Clinical signs of neurotoxicity
Lowest relevant oral NOAEL	2.2 mg/kg bw per day (90-day study in dogs)
Lowest relevant dermal NOAEL	20 mg/kg bw per day (21-day study in rabbits)
Lowest relevant inhalation NOAEL	0.050 mg/l (21-day study in rats)
Genotoxicity	
	No genotoxic potential
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body-weight gain and food consumption
Lowest relevant NOAEL	7.5 mg/kg bw per day (2-year dietary study in rats)
Carcinogenicity	Not carcinogenic in rats and mice
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects; decreased pup body weight
Lowest relevant reproductive NOAEL	6 mg/kg bw per day (rats)
Developmental target/critical effect	Decreased fetal weights (rats)
Lowest relevant developmental NOAEL	9 mg/kg bw per day
Neurotoxicity	
Target/critical effect	Clinical signs, changes in FOB tests and degenerative changes to the sciatic nerve
Lowest relevant NOAEL	4 mg/kg bw per day (single-dose study in rats)
Delayed neurotoxicity	
Target/critical effect	No delayed effect
Lowest relevant NOAEL	> 1000 mg/kg bw per day (hens)
Medical data	Paraesthesia after dermal exposure

Summary for cypermethrins, including alpha-cypermethrin and zeta-cypermethrin

	Value	Study	Safety factor
Group ADI	0–0.02 mg/kg bw per day	Dog, 3-month dietary study with alpha-cypermethrin	100
Group ARfD	0.04 mg/kg bw	Rat, study of acute neurotoxicity with alpha-cypermethrin	100

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CYROMAZINE

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Explanation

Cyromazine, the International Organization of Standardization (ISO) approved name for ***N***-cyclopropyl-[1,3,5] triazine-2,4,6-triamine, is a selective insecticide used on a broad range of vegetable crops. It acts by inhibiting the moulting process, particularly in dipterian insects.

Cyromazine was first evaluated by the 1990 JMPR, when an acceptable daily intake (ADI) of 0–0.02 mg/kg bw was established on the basis of a no-observed-adverse-effect level (NOAEL of 1.8 mg/kg bw per day for body-weight changes in a 2-year dietary study in rats and a NOAEL of 2 mg/kg bw per day in a two-generation study of reproductive toxicity in rats, with a safety factor of 100.

Cyromazine was considered by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR). The Meeting reviewed new data on cyromazine, including studies of toxicokinetics, metabolism, dermal absorption, acute toxicity after inhalation, skin sensitization, a 1-year study of toxicity in dogs and a 3-week study of dermal toxicity in rabbits, as well as data on mutagenesis and toxicity for the metabolite, melamine. Relevant data from the previous evaluation were also considered.

All pivotal studies with cyromazine were certified as complying with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

The excretion and tissue distribution of radioactivity was investigated in male and female Charles River albino rats given a single oral dose of [U-^{14}C triazine]cyromazine at 0.5 mg/kg bw (specific activity, 1.395 MBq/mg; radiochemical purity, > 99%) in aqueous Carbowax 400 formulation. Exhaled volatile metabolites were collected from one male and one female housed individually in glass metabolism cages. One female and two males were housed singly in metal metabolism cages for 72 h for the collection of urine and faeces. Seventy-two hours after dosing, the rats were killed and a range of tissues was taken for the measurement of radioactivity. This comprised brain, fat, heart, kidneys, liver, lungs, muscle, ovaries, spleen, testes and whole blood. All samples were counted for radioactivity by liquid scintillation counting (LSC), either directly or after sample oxidation. The study was performed before the introduction of GLP and prior to OECD TG 417 (1984), but complied to a great extent with these requirements.

The predominant route of excretion was via the kidneys, with approximately 94% or more of the administered dose excreted in the urine (Table 1). Hence, after an oral dose at 0.5 mg/kg bw, absorption appeared to be almost quantitative. Faecal elimination accounted for just 4% and 1% of the administered dose in males and females respectively. Virtually no radioactivity was recovered from exhaled air. Excretion was rapid since greater than 97% of the administered dose was excreted within 24 h. The urinary and faecal excretion data were derived from the rats housed in metal metabolism cages. The exhaled volatile radioactivity measurements were derived from the rats housed in glass metabolism cages.

Table 1. Excretion profiles over 72 h in rats given [U-^{14}C triazine]cyromazine as a single oral dose at 0.5 mg/kg bw

Excretion	Percentage of administered dose	
	Male (n = 2)	Female (n = 1)
Urine		
0–24 h	93.8	96.6

24–48 h	< 0.3	0.5
48–72 h	< 0.2	0.3
Subtotal	93.8	97.4
Faeces		
0–24 h	3.8	0.6
24–48 h	< 0.1	0.4
48–72 h	< 0.1	< 0.1
Subtotal	3.8	1.0
Cage wash	< 0.1	< 0.1
Volatile metabolites	< 0.1	< 0.1
Exhaled carbon dioxide	< 0.1	< 0.1
Tissues	< 0.1	< 0.1
Blood	< 0.1	< 0.1
Intestinal tract	< 0.1	< 0.1
Total recovery	97.5	98.4

From Simoneaux & Cassidy (1978)

Three days after oral dosing, tissue residues were very low, with < 0.007 ppm in the liver and < 0.003 ppm in all other tissues. This was consistent with the rapid and extensive excretion of the administered dose. The tissue residue data tabulated were derived from the rats housed in metal metabolism cages.

A single oral dose of [U-^{14}C triazine]cyromazine at 0.5 mg/kg bw was almost quantitatively absorbed and was rapidly excreted, almost exclusively in the urine. More than 97% of the administered dose was eliminated within 24 h after dosing and 72 h after dosing, tissue residues were very low (Simoneaux & Cassidy, 1978).

The excretion and tissue distribution of [U-^{14}C triazine]cyromazine was investigated in male and female Sprague Dawley rats (Table 2).

Table 2. Design of a study to investigate excretion and tissue distribution of [U-^{14}C triazine] cyromazine in male and female rats

Group	Number and sex	Route and dose of [U-^{14}C triazine]cyromazine
1	One male and one female	Single intravenous dose of vehicle (carboxymethylcellulose plus Hi Sil)
2	Five males and five females	Single intravenous dose at approx. 3 mg/kg bw
3	Five males and five females	Single oral dose at approx. 3 mg/kg bw
4	Five males and five females	Single oral dose at approx. 3 mg/kg bw. Rats pre-conditioned with 14 daily non-radiolabelled doses at 3 mg/kg bw.
5	Five males and five females	Single oral dose at approx. 300 mg/kg bw
6	One male and one female	Single oral dose of vehicle
7	One male and one female	Rats dosed with vehicle each time group 4 received a dose.
8	One male and one female	Single oral dose of dose vehicle

From Capps (1990)
Approx., approximately.

After each dose of [U-^{14}C triazine]cyromazine (specific activity, 9.8 µCi/mg for the lowest dose and 0.8 µCi/mg for the highest dose; radiochemical purity, 97.2%), urine and faeces were collected at intervals over 7 days from rats in groups 2, 3, 4 and 5. At termination, samles of a range of tissues

were taken for the measurement of radioactivity present. These comprised bone (femur), brain, carcass, fat, heart, kidneys, liver, lungs, muscle, ovaries, plasma, erythrocytes, spleen, testes and uterus. Radioactivity was measured by LSC, either directly or after sample combustion. The study was conducted according to the principles and practices of GLP (including certification for quality assurance, QA) and the protocol was in accordance with OECD TG 417 (1984).

The predominant route of excretion was via the kidneys, irrespective of the route of administration. The inclusion of radioactivity from cage rinses and washes with urine shows that 82–92% of the administered dose was attributable to urinary excretion over 7 days (Table 3). Hence, absorption of an oral dose of cyromazine was extensive. Faecal elimination over the same period accounted for between 3% and 8% of the dose. There was no apparent sex difference in excretion profiles, although there were some minor differences in the rates of elimination. Excretion of radioactivity in urine and faeces over the first 24 h after dosing was slightly faster in rats given a single oral dose at 3 mg/kg bw compared with rats given a single oral dose at 300 mg/kg bw.

Table 3. Excretion data in rats given [U-^{14}C triazine]cyromazine as a single oral dose at 3 or 300 mg/kg bw

Sample	Percentage of administered dose							
	Group 2		Group 3		Group 4		Group 5	
	Single intravenous dose		Single oral dose		Single oral dose (pre-treated for 14 days)		Single oral dose	
	Male	Female	Male	Female	Male	Female	Male	Female
	Dose (mg/kg bw)							
	3		3		3		300	
Urine								
0–24 h	78.14	72.38	65.22	56.87	77.82	58.55	51.81	55.64
24–48 h	1.58	0.90	1.22	2.48	0.80	2.53	10.10	9.75
48–168 h	1.09	1.50	1.70	3.53	2.20	2.13	3.82	2.52
Subtotal	80.81	74.78	68.14	62.88	80.82	63.21	65.73	67.91
Cage rinse								
24 h	1.71	8.79	8.38	14.8	9.36	24.9	15.20	14.60
Cage wash								
168 h	2.96	1.83	4.24	6.40	0.81	0.93	1.56	2.89
Cage wipe								
168 h	1.08	1.09	1.61	2.34	0.89	1.05	1.02	1.04
Subtotal	5.75	11.71	14.23	23.54	11.06	26.88	17.78	18.53
Faeces								
0–24 h	2.62	4.80	3.24	2.04	1.40	1.34	3.96	2.73
24–48 h	1.16	0.68	0.35	0.79	0.33	0.34	2.20	1.69
48–168 h	1.39	0.96	0.47	0.92	1.57	1.02	1.36	1.93
Subtotal	5.17	6.44	4.06	3.75	3.30	2.70	7.52	6.35
Excretion summary								
0–24 h	82.47	85.97	76.84	73.71	88.58	84.79	70.97	72.97
24–48 h	2.74	1.58	1.57	3.27	1.13	2.87	12.30	11.44
Total excretion	*91.73*	*92.93*	*86.43*	*90.17*	*95.18*	*92.79*	*91.03*	*92.79*

From Capps (1990)
Values are expressed as percentage of dose

Seven days after an oral dose of [U-^{14}C triazine]cyromazine, tissue residues were detectable only in carcass (at 0.004–0.017 ppm after the lowest dose and 0.565–0.782 ppm after the highest dose), liver (at 0.004–0.011 ppm after the lowest dose and 0.455–0.601 ppm after the highest dose) and erythrocytes (at < 0.001–0.001 ppm after the lowest dose and 0.153–0.164 ppm after the highest dose) of rats at 3 or 300 mg/kg bw and in the spleen (< 0.001 ppm) of female rats at 300 mg/kg bw. Tissue residue concentrations were very low, < 0.017 ppm at 3 mg/kg bw and < 0.79 ppm at 300 mg/kg bw.

A single oral dose of [U-^{14}C triazine]cyromazine at 3 mg or 300 mg/kg bw was extensively and rapidly absorbed in male and female rats. At both doses, excretion was rapid and predominately in the urine. Seven days after dosing, tissue residues were very low and detectable only in carcass, liver, erythrocytes and spleen. There were no pronounced differences between the sexes and relatively minor differences between the doses. The pretreatment of rats with unlabelled cyromazine (3 mg/kg bw per day) for 14 days before a single radiolabelled dose of 3 mg/kg bw had no marked effect on its absorption or excretion (Capps, 1990).

The blood kinetics, tissue distribution and tissue depletion of radioactivity were studied in male and female Sprague-Dawley rats given [U-^{14}C triazine]cyromazine as a single oral dose at 3 mg or 300 mg (specific activity, 1.87 MBq/mg for the lowest dose and 36.67–46.14 kBq/mg for the highest dose; radiochemical purity, > 97%)/kg bw in ethanol : PEG 200 : water (2 : 5 : 3 v/v).

Table 4. Design of a study to investigate blood kinetics, tissue distribution and tissue depletion in rats given [U-^{14}C triazine]cyromazine as a single oral dose

Group	Number and sex	Regime
E1	Three males	Approximately 3 mg/kg bw with collection of blood over a time course
E2	Three males	Approximately 300 mg/kg bw with collection of blood over a time course
E3	Three females	Approximately 3 mg/kg bw with collection of blood over a time course
E4	Three females	Approximately 300 mg/kg bw with collection of blood over a time course
F1	Twelve males	Approximately 3 mg/kg bw with collection of tissues over a time course
F2	Twelve males	Approximately 300 mg/kg bw with collection of tissues over a time course

From Paul & Dunsire (1994)

Blood was collected from rats in groups E1 to E4 at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 32, 48, 72, 96 and 120 h after dosing. Based upon the profiles determined for groups E1 and E3, rats in groups F1 and F2 were killed in groups of three over a time course (F1, 0.5, 3.5, 5.5 and 24 h after dosing; F2, 8, 21, 27 and 48 h after dosing) and samples of a range of tissues were taken for the measurement of radioactivity present. These comprised heart, lungs, spleen, liver, abdominal fat, skeletal muscle, brain, bone, testes, kidney, urinary bladder, plasma, erythrocytes, gastrointestinal tract and contents and residual carcass. Radioactivity was measured by LSC, either directly or after sample combustion. The study was conducted according to the principles and practices of GLP (with QA certificate) and the protocol was in accordance with OECD TG 417 (1984).

Blood kinetics data show that after a dose at 3 mg/kg bw, concentrations of radiolabel rose quickly, reaching a maximum at 0.5 h after dosing (Table 5). By 8 h after dosing, blood concentrations had declined markedly and by 24 h approached background values. Elimination appeared to be biphasic, with an initial rapid phase of 2–12 h, followed by a slower phase. In males given a dose at 300 mg/kg bw, two peaks were present, at 0.5 and 8 h. After 24 h, [U-^{14}C triazine] concentrations declined rapidly. In female rats at 300 mg/kg bw, concentrations of the radiolabel rose to a broad plateau between 1 h and 4 h, declining quickly after 12 h. The plasma data showed that the area under the curve of concentration-time (AUC, 0–24 h) increased approximately 150-fold after a 100-fold increase in dose, reflecting the sustained high blood concentrations, particularly at the

highest dose, rather than indicating that the extent of absorption was dose-dependent. Similarly, there was no evidence to suggest that blood kinetics were sex-dependent, hence only male rats were used to investigate the distribution and elimination of radioactivity from tissues.

Table 5. Group mean blood kinetics in rats given [U-^{14}C triazine]cyromazine as a single oral dose

Time (h)	Radiolabel (ppm cyromazine equivalents)			
	Dose (mg/kg bw)			
	Approx. 3	Approx. 300	Approx. 3	Approx. 300
	Group E1[a]	Group E2[a]	Group E3[a]	Group E4[a]
	Male	Male	Female	Female
0.25	0.743	20.4	0.967	31.0
0.50	1.151	23.5	1.059	35.9
1	1.025	21.3	0.897	38.5
2	0.775	19.1	0.757	45.4
4	0.469	27.3	0.373	42.7
8	0.040	34.8	0.057	34.0
12	0.007	28.4	0.015	31.7
24	0.005	13.9	0.008	10.5
32	0.005	0.5	0.004	1.0
48	0.004	0.3	0.005	0.3
72	0.002	0.4	0.001	0.2
96	0.003	0.3	0.004	0.2
120	0.002	0.2	0.002	0.1
AUC 0–24 h[b]	4.2	590	3.9	697

From Paul & Dunsire (1994)

[a] $n = 3$.

[b] AUC 0–24 h expressed as ppm × h.

At the first three time-points, the highest concentrations of radioactivity to male rats given a single oral dose of[U-^{14}C triazine]cyromazine at 3 mg/kg bw were present in the gastrointestinal tract and its contents (21.352, 5.379 and 0.962 ppm, respectively), the bladder (2.468, 3.523 and 2.359 ppm, respectively), kidneys (1.955, 2.090 and 0.790 ppm, respectively) and liver (0.859, 0.913 and 0.409 ppm, respectively). The radiolabel found in bladder was attributed to residues in urine, since it has been established that urine is an important route of elimination of cyromazine metabolites. Twenty-four hours after dosing, all tissue residues were very low and, with the exception of the gastrointestinal tract and its contents (0.010 ppm), liver (0.061 ppm), kidney (0.002 ppm) and erythrocytes (0.005 ppm), were approaching background values. Assuming first-order kinetics, the elimination half-life between 3.5 and 24 h was approximately 6 h for liver, compared with approximately 2 h for other tissues. The Meeting concluded that there was no tissue accumulation of radioactivity.

At the first three time-points, the highest concentrations of radioactivity in male rats given a single oral dose of [U-^{14}C triazine]cyromazine at 300 mg/kg bw were present in the gastrointestinal tract and its contents (1233, 128 and 22 ppm, respectively), the bladder (196, 223 and 23 ppm, respectively), kidneys (83, 51 and 16 ppm, respectively) and liver (49, 24 and 10 ppm, respectively). As for the lowest dose, the radiolabel found in bladder was attributed to residues in urine. Forty-eight hours after dosing, all tissue residues were very low, < 0.3 ppm, with the single exception of liver (2.23 ppm) and gastrointestinal tract and its contents (0.66 ppm). Assuming first-order

kinetics, the elimination half-life between 21 and 48 h was approximately 8 h for liver, compared with approximately 3–5 h for other tissues. The Meeting concluded that there was no tissue accumulation of radioactivity.

After a single oral administration of [U-^{14}C triazine]cyromazine at 3 mg or 300 mg/kg bw in male and female rats, absorption was very rapid. Overall, rapid elimination of residues from tissues occurred. Blood kinetic data in both sexes and tissue depletion data in males suggested that the rates of distribution and/or elimination were rate-limiting. It was assumed that blood kinetics and tissue distribution were independent of sex and dose (Paul & Dunsire, 1994).

The absorption, distribution and excretion of cyromazine was investigated in male rats given radiolabelled cyromazine as 14 consecutive doses at 3.0 mg/kg bw per day by oral gavage. A group of 16 male Hanlbm:WIST (SPF) rats (Group J1) was given [U-^{14}C triazine]cyromazine (specific activity, 2580 kBq/mg; radiochemical purity, ≥ 98.5%) dissolved in a mixture of ethanol/polyethylene glycol 200/water (2/5/3, v/v). The depletion of radioactivity was measured for 5 days after the last dose. At each time-point, three animals were selected and killed. The residual radioactivity in selected tissues (adrenals, blood, bone, brain, fat, heart, kidneys, liver, lungs, muscle, pancreas, plasma, spleen, testes, thymus, thyroids) was determined during the administration phase on days 1 (T1), 7 (T2), 14 (T3) after the first dose and during the depletion phase on day 18 (T4) after the first dose. Serial daily blood samples were collected from rats in the subgroup treated for 14 days. The excreted radioactivity was determined in the urine and faeces at daily intervals. Radioactivity was measured by LSC. Plasma and urine were measured directly. Aliquots of adrenals, brain, fat, heart, kidneys, liver, muscle, pancreas, spleen, testes, thymus, and thyroids were digested with Soluene 350 tissue-solubilizer, and neutralized with hydrochloric acid before measuring the radioactivity by LSC. Radioactivity in blood, bone, faeces, lung and carcass was measured by combustion analysis. The stability of the test substance in the administered solution was checked by thin-layer chromatography (TLC) at the time of the first, eighth and after the last dose. Cyromazine was stable over the dosing period and represented more than 96% of the radioactivity. The study was conducted according to the principles and practices of GLP (with QA certificate) and the protocol was in accordance with OECD TG 417 (1984).

The test substance was rapidly absorbed from the gastrointestinal tract into the systemic circulation and the daily doses were rapidly and almost completely excreted within 24 h after dosing. After the start of dosing, a steady state in terms of excretion was reached within 1 day. Most of the daily administered dose was excreted via the urine (about 90%) and a smaller part was excreted via the faeces (about 4%). In total, 92.9% and 4.2% of the administered dose was excreted via the urine and the faeces, respectively (Table 6). One and five days after the last dose, only 0.01% and less than 0.01%, respectively, of the total administered dose remained in tissues and organs.

Table 6. Excretion and recovery of radiolabel in male rats given 14 consecutive daily oral doses of [U-^{14}C triazine]cyromazine at 3 mg/kg bw by gavage

Sample	Percentage of total administered dose	
	Day 18	
	Mean	Standard deviation
Urine	92.86	0.61
Faeces	4.19	0.71
Cage wash	0.16	0.06
Total excretion, 0–18 days	97.21	0.67

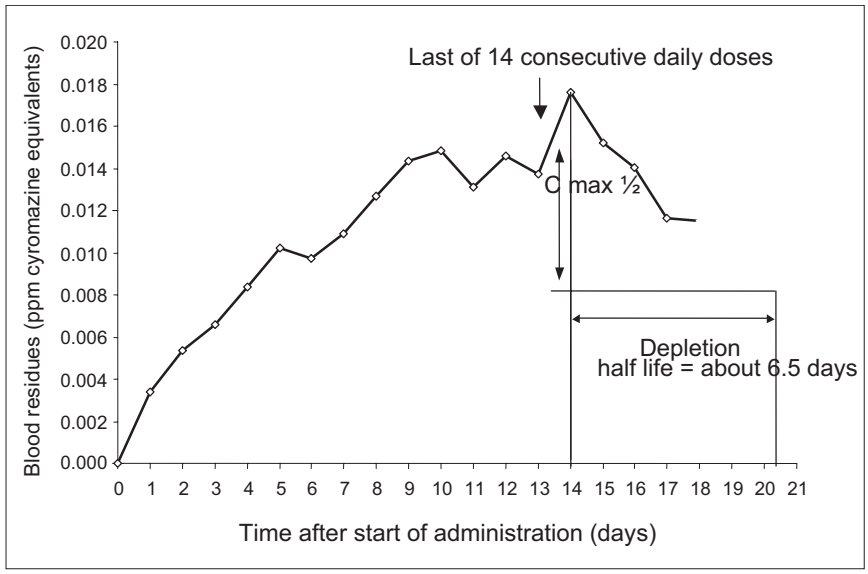
Tissue residues:		
Tissues[a]	< 0.01	< 0.01
Carcass	0.17	0.02
Subtotal	0.18	0.02
Total recovery	97.39	0.68

From Löffler (2003)

[a] Residues determined in the excised part of the tissues and organs.

Serial blood residue concentrations were determined at 24 h intervals, before dosing and starting 1 day after the first administration. The blood kinetics showed increasing residue values with ongoing administration, reaching a plateau of about 0.016 ppm expressed as cyromazine equivalents within about 9 days (Figure 1). After the last dose, the very low residues in whole blood decreased with a half-life of about 6.5 days.

Figure 1. Blood residue concentrations at different time-points in rats given radiolabelled cyromazine by gavage for 14 days



From Löffler (2003)

The very low concentrations of tissue residues determined at four different time-points during and after the dosing period showed increasing residue concentrations for all of the tissues and organs during the dosing period, reaching their maximum 1 day after the last dose. Apparently, the residues in liver reached a plateau within the dosing period, while the other tissues showed a slow increase with ongoing administration. Highest maximum residue concentrations were determined in liver (0.080 ppm) and kidneys (0.024 ppm), followed by whole blood (0.015 ppm), adrenals (0.015 ppm) and thyroids (0.014 ppm). All other tissues and organs showed very low maximum concentrations not exceeding 0.01 ppm expressed as cyromazine equivalents.

After reaching the maximum concentration, the residues declined in all tissues and organs to at least half of the maximum concentration within 3.4 days after the last dose, except for whole blood. The calculated half-life (t½) for the depuration ranged from 1.9 (plasma) to 6.4 days (whole blood).

The metabolite pattern for urine and faeces, investigated at three different time intervals during dosing, was not influenced by repeated doses. In total, about 85% of the daily dose was excreted as unchanged parent via urine (83%) and faeces (2%).

Cyromazine was rapidly absorbed and rapidly and almost completely excreted after repeated doses. As observed after a single dose, the tissue residue concentrations were very low. A plateau for tissue residues was reached only in liver. All other tissue residues increased with ongoing dosing and did not reach a plateau within the 14 day dosing period. The rates and routes of excretion after repeated doses were similar to those after a single dose (Löffler, 2003).

Monkeys

A study was performed to investigate the excretion pattern of cyromazine in monkeys. Two male and two female (*Macaca fasicicula*) monkeys were given a single oral dose of [U-^{14}C triazine] cyromazine at 0.05 mg/kg bw. An additional two male and two female monkeys were given a single oral dose of [U-^{14}C triazine]cyromazine at 0.5 mg/kg bw. Each animal weighed approximately 3 kg. The doses were administered in capsules containing [U-^{14}C triazine]cyromazine (specific activity, 16.6 µCi/mg; radiochemical purity, > 97.5%) on ground corn-on-the-cob. Each monkey was housed in a metabolism cage to which it had been first acclimatized. Urine and faeces were collected for 24 h before dosing and at daily intervals after dosing for 4 days. Radioactivity in urine and in combusted samples of faeces was measured by LSC. The study was not performed according to GLP, but it was an investigative study designed to complement the regulatory submission on metabolism. Tissue residues were not investigated. The study was regarded as additional information for the evaluation owing to incomplete recoveries of radioactivity.

At both doses, cyromazine was rapidly and extensively absorbed, as most of the administered radioactivity was excreted in the urine within 1 day of dosing (Table 7). At 0.05 mg/kg bw, males excreted 73% of the administered radioactivity in the urine and approximately 1% in the faeces over 4 days; females excreted 82% in urine and 1% in faeces over the same period. At 0.5 mg/kg bw, males excreted 56% of administered radioactivity in the urine and 1.5% in faeces over 4 days; females excreted 80% in the urine and less than 2% in faeces over the same time interval. The incomplete recoveries of administered dose were attributed to losses in the cages as no cage washes were collected. No tissue residues were measured.

Table 7. Excretion data in monkeys given [U-^{14}C triazine]cyromazine as a single oral dose at 0.05 or 0.5 mg/kg bw

Time-point	Percentage of administered dose							
	Dose (mg/kg bw)							
	0.05		0.5		0.5		0.5	
	Male		Female		Male		Female	
	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
Day –1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	69.69	0.61	80.83	0.56	52.22	1.06	78.01	0.85
2	2.81	0.55	1.00	0.69	3.27	0.39	1.68	0.69
3	0.46	0.07	0.18	0.05	0.26	0.02	0.20	0.19
4	0.00	0.00	0.16	0.00	0.09	0.01	0.07	0.03
Subtotal	72.96	1.23	82.16	1.29	55.84	1.48	79.95	1.75
Total excretion	74.19		83.45		57.32		81.70	

From Staley (1986)

In male and female monkeys, a single oral dose of [U-^{14}C triazine]cyromazine at 0.05 or 0.5 mg/kg bw was rapidly and extensively absorbed and rapidly excreted, predominantly in the urine (Staley, 1986).

Because of the low total recovery of radioactivity in the above study, a second study was performed in one male and one female of the same strain of monkey and at the same doses. Each animal weighed approximately 3 kg. The doses were administered in capsules containing [U-^{14}C triazine]cyromazine (specific activity, 16.6 μCi/mg; radiochemical purity, > 97.5%) on ground corn-on-the-cob. Each monkey was housed in a metabolism cage to which it had been first acclimatized. Urine and faeces were collected for 24 h before dosing and at daily intervals after dosing for 2 days. Radioactivity in the urine and in combusted samples of faeces was measured by LSC. The study was not performed according to GLP, but it was an investigative study designed to complement the regulatory submission on metabolism. Tissue residues were not investigated. The study was regarded as additional information for the evaluation owing to incomplete recoveries of radioactivity.

At both doses, cyromazine was rapidly and extensively absorbed, as most of the administered radioactivity was excreted in the urine within 1 day of dosing (Table 8). At 0.05 mg/kg bw, the male excreted 34% of administered radioactivity in urine with an additional 10% in the cage wash and approximately 13% in the faeces over 2 days; the female excreted 50% in the urine with an additional 15% in the cage wash and 15% in the faeces over the same time interval. At 0.5 mg/kg bw, the male excreted 65% of administered radioactivity in the urine with an additional 11% in the cage wash and only 0.3% in the faeces over 2 days; the female excreted 51% in the urine with an additional 8% in the cage wash and less than 0.1% in the faeces over the same period. The incomplete recoveries of administered radioactivity could not be explained, particularly since cage washes were collected during this study. No tissue residues were measured.

In male and female monkeys, a single oral dose of [U-^{14}C triazine]cyromazine at 0.05 or 0.5 mg/kg bw was rapidly and extensively absorbed and rapidly excreted, predominantly in the urine (Staley & Simoneaux, 1986).

Table 8. Excretion data in monkeys given [U-^{14}C triazine]cyromazine as a single oral dose at 0.05 or 0.5 mg/kg bw

Time-point	Percentage of administered dose											
	Dose (mg/kg bw)											
	0.05						0.5			0.5		
	Male			Female			Male			Female		
	Urine	Faeces	Cage wash	Urine	Faeces	Cage wash	Urine	Faeces	Cage wash	Urine	Faeces	Cage wash
Day –1	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	32.33	1.08	9.40	48.77	13.99	14.00	64.96	0.24	11.34	50.57	0.05	7.93
2	1.44	12.14	0.44	0.85	0.93	1.02	0.42	0.07	0.10	0.86	0.00	0.10
Subtotal	34.10	13.22	9.84	49.62	14.92	15.02	65.38	0.31	11.44	51.43	0.05	8.03
Total excretion	57.16			79.56			77.13			59.51		

From Staley & Simoneaux (1986)

Goats

Lactating goats received [U-^{14}C triazine]cyromazine at a dose equivalent to a dietary concentration of 4.6 and 48.4 ppm for 10 days.

Most of the administered dose of [U-^{14}C triazine]-cyromazine was excreted in the urine (82–90%) and 6–7% in faeces. A small proportion was found in blood (0.3%), tissues (0.8–1.8%),

gastrointestinal tract (1.1–1.3%) and milk (0.3–0.4%). Cyromazine was thus rapidly eliminated from goats over a 10-day feeding period and did not accumulate in body tissues or blood.

Radioactivity in blood and milk reached a plateau by the second day of feeding at both doses. The goat at the lowest dose had average values of 0.017 mg/kg (as cyromazine) in milk and 0.012 mg/kg (as cyromazine) in blood while the animal at the highest dose had average values of 0.318 mg/kg in milk and 0.136 mg/kg in blood, indicating an approximate proportionality between dose and residue concentration. Radioactivity in the tissues of the goat at the lowest dose was ≤ 0.01 mg/kg except for kidney (0.04 mg/kg) and liver (0.79 mg/kg); that in the tissues of the goat at the highest dose was ≤ 0.15 mg/kg, except for kidney (0.44 mg/kg) and liver (1.52 mg/kg).

In conclusion, at least 80% of the administered dose of cyromazine was absorbed from the gastrointestinal tract and rapid and complete excretion occurred mainly via the urine. As a consequence, low concentrations of residues were detected in milk and tissues, and there was no evidence of accumulation or retention (Simoneaux & Marco, 1984). This study had been evaluated by the 1990 JMPR and was not reviewed by the present Meeting.

Hens

Two hens received capsules containing [U-^{14}C triazine]cyromazine at a dose equivalent to a dietary concentration of 5.0 ppm for 7 days.

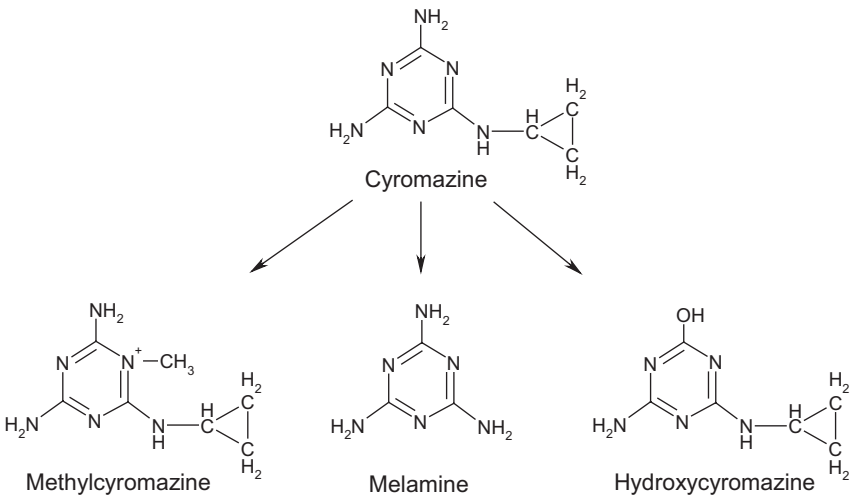
Most (99%) of the administered cyromazine dose was recovered from the excreta of the hens. Egg white and yolk contained 0.4% and 0.2% of the administered dose respectively. Tissue concentrations were low (≤ 0.05 mg/kg, as cyromazine) (Simoneaux & Cassidy, 1979). This study had been evaluated by the 1990 JMPR and was not reviewed by the present Meeting.

1.2 Biotransformation

Rats

A generalized metabolic pathway for cyromazine in rats is shown in Figure 2.

Figure 2. Proposed metabolic pathway of cyromazine in rats



Name	Description	Compound found in:	Denomination
Cyromazine	*N*-cyclopropyl-[1,3,5] triazine-2,4,6-triamine	Rat, monkey, celery, lettuce, tomatorotational celerygoathen eggs and liver	Parent compound
Melamine	*N*-cyclopropyl-[1,3,5] triazine-2,4,6-triamine	Rat, monkey, celery, lettuce, tomatorotational celerygoathen eggs and liver	Major metabolite
N-methyl cyromazine,1-methyl cyromazine	2,4-diamino-6-cyclo-propylamino-1-methyl-[1,3,5]triazin-1-ium salt	Rat, goat	—
Hydroxy-cyromazine	—	Rat	—

In the pharmacokinetic study in rats given a single oral dose of [U-^{14}C triazine]cyromazine at 0.5 mg/kg bw, metabolite profiles in the urine and faeces were investigated by TLC. Faecal samples were extracted with acetonitrile : water (9 : 1), which extracted more than 90% of faecal radioactivity.

Urine contained predominantly unchanged parent, which accounted for approximately 80% of the dose and three minor metabolites each of which represented less than 5% of the administered dose. Faecal metabolite profiles appeared to be similar to those for urine, but from the low concentrations of faecal radioactivity, it was not possible to confirm that the metabolites corresponded to those excreted in urine.

Cyromazine was poorly metabolized since more than 80% of the administered dose was excreted unchanged (Simoneaux & Cassidy, 1978).

A study was performed to determine whether cyromazine (*N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine) was metabolized to melamine (1,3,5-triazine-2,4,6-triamine) in rats. One male and one female fasted Sprague Dawley rat were fed diets containing cyromazine (purity, > 96.5%) at a concentration of 3000 ppm for 10 days. The rats were housed in metabolism cages from which urine and faeces were collected, although these samples were not analysed. The rats were then killed and the liver and kidneys were removed and analysed for cyromazine and melamine residues. Homogenates of both tissues were extracted with 90% acetonitrile : water, filtered and separated by ion exchange before high-performance liquid chromatography with ultraviolet light detector (HPLC-UV) determination of the relative proportions of cyromazine and melamine. Samples of treated diet were similarly analysed to determine the amount of melamine, originating from technical-grade cyromazine, which is known to contain melamine as a manufacturing impurity. The study was not performed according to GLP but it was an investigative study designed to complement the regulatory submission on metabolism.

The diet was shown to contain the correct amount of test substance. The ratio of cyromazine to melamine in diet was 120 : 1 (Table 9).

Table 9. Analysis of diet for cyromazine and melamine in a study in rats fed diets containing cyromazine at a concentration of 3000 ppm for 10 days

Sample	Cyromazine (ppm)	Melamine (ppm)	Ratio Cyromazine/melamine
Diet containing cyromazine on day 0	2585	25	103 : 1
Diet containing cyromazine on day 10	2933	21	140 : 1
Control diet	< 1.0	< 1.0	—

From Smith et al. (1983)

Residue concentrations of cyromazine and melamine were higher in the kidneys than in the liver and higher in males than in females (Table 10). The mean ratios of cyromazine : melamine between the sexes were approximately 30 : 1 for liver and approximately 40 : 1 for kidney. These ratios are quite different from those in the administered diet (120 : 1). Hence, these data indicate that rats metabolized cyromazine to melamine in liver and kidney.

Table 10. Analysis of liver and kidney for cyromazine and melamine in rats fed diets containing cyromazine at a concentration of 3000 ppm for 10 days

Sample	Cyromazine (ppm)	Melamine (ppm)[a]	Ratio
Liver			
Male liver at day 10 on control diet	< 0.05	< 0.05	—
Male liver at day 10 on diet containing cyromazine	31.3	0.96	33 : 1
Female liver at day 10 on diet containing cyromazine	13.2	0.51	26 : 1
Kidney			
Male kidney at day 10 on control diet	< 0.05	< 0.05	—
Male kidney at day 10 on diet containing cyromazine	62.4	1.3	48 : 1
Female kidney at day 10 on diet containing cyromazine	22.2	0.68	33 : 1

From Smith et al. (1983)

[a] Melamine residues have been converted to cyromazine equivalents.

Male and female rats were fed diets containing technical-grade cyromazine (containing melamine as a 0.8% impurity) at a concentration of 3000 ppm. After 10 days feeding, residues of cyromazine and melamine were higher in the kidneys than in the liver and higher in tissues in males than in females. The mean ratios of cyromazine : melamine between the sexes were approximately 30 : 1 for liver and approximately 40 : 1 for kidney, indicating that rats can metabolize cyromazine to melamine (Smith et al., 1983).

The biotransformation of [U-^{14}C triazine]cyromazine was investigated in male and female Sprague Dawley rats given doses as described in Table 11.

Table 11. Doses administered to male and female rats in a study on biotransformation of [U-^{14}C triazine]cyromazine

Group	Number and sex	Route and dose of [U-^{14}C triazine]cyromazine
1	One male and one female	Single intravenous dose of vehicle (carboxymethylcellulose plus Hi Sil)
2	Five males and five females	Single intravenous dose at approx. 3 mg/kg bw
3	Five males and five females	Single oral dose at approx. 3 mg/kg bw
4	Five males and five females	Single oral dose at approx. 3 mg/kg bw. Rats first conditioned with 14 daily non-radiolabelled doses at 3 mg/kg bw.
5	Five males and five females	Single oral dose at approx. 300 mg/kg bw
6	One male and one female	Single oral dose of vehicle
7	One male and one female	Rats dosed with vehicle each time group 4 received a dose.
8	One male and one female	Single oral dose of vehicle

From Capps (1990)
Approx., approximately.

After each dose of [U-^{14}C triazine]cyromazine (specific activity, 9.8 µCi/mg for the lowest dose and 0.8 µCi/mg for the highest dose; radiochemical purity, 97.2%), urine and faeces were collected at intervals over 7 days from rats in groups 2, 3, 4 and 5. Metabolite profiles in urine (collected over 0–24 h after a dose at 3 mg/kg bw and over 0–36 h after a dose at 300 mg/kg bw) and in solvent extracts of faeces (collections over 0–72 h) were investigated by TLC and liquid chromatography (LC). Structural assignments of resolved metabolites were confirmed by mass spectrometry. The study was conducted according to the principles and practices of GLP (with QA certificate) and the protocol was in accordance with OECD TG 417 (1984).

The major component present in urine was cyromazine, accounting for 72% of urinary radioactivity (Table 12). An additional 7% was attributable to melamine, 9% to hydroxy-cyromazine and 2% to methyl-cyromazine, although there was no detectable amount of this latter metabolite in urine from rats at 300 mg/kg bw. Only 6% of [U-^{14}C triazine]-metabolites in urine remained uncharacterized and comprised minor metabolites, each of which represented less than 2% of urinary radioactivity. The urinary metabolite profiles were similar between the sexes and, apart from the absence of methyl-cyromazine in the group at the highest dose, there were no pronounced differences between doses.

Table 12. Quantification of urinary metabolites in rats given a single oral dose of [U-^{14}C triazine] cyromazine at 3 or 300 mg/kg bw

Metabolite	Percentage of administered radioactivity							
	Group 2		Group 3		Group 4		Group 5	
	Single intravenous dose		Single oral dose		Single oral dose (pre-treated for 14 days)		Single oral dose	
	Dose (mg/kg bw)							
	3		3		3		300	
	Male	Female	Male	Female	Male	Female	Male	Female
Methyl-cyromazine	1.98	2.93	2.15	2.02	1.63	1.59	ND	ND
Unidentified	5.19	6.11	6.54	6.76	6.60	6.09	2.59	1.73
Melamine	5.27	7.20	6.54	7.15	7.05	10.68	3.48	2.31
Hydroxy-cyromazine	6.76	5.69	14.02	8.47	8.32	4.06	4.94	4.86
Cyromazine	58.92	59.26	54.35	50.82	63.82	61.63	67.63	68.64

From Capps (1990)

ND, not detected.

The chromatographic profile of metabolites in faecal extracts was similar to that in urine with 71% of radioactivity corresponding to cyromazine and 7% to melamine (Table 13). An average of 8% co-chromatographed with methyl-cyromazine and hydroxy-cyromazine and 13% did not chromatograph with available standards and appeared to comprise several minor metabolites. The faecal metabolite profiles were similar for male and female rats.

Table 13. Quantification of faecal metabolites in rats given a single oral dose of [U-^{14}C triazine] cyromazine at 3 or 300 mg/kg bw

Metabolite	Percentage of administered radioactivity							
	Group 2		Group 3		Group 4		Group 5	
	Single intravenous dose		Single oral dose		Single oral dose (pre-treated for 14 days)		Single oral dose	
	Dose (mg/kg bw)							
	3		3		3		300	
	Male	Female	Male	Female	Male	Female	Male	Female
Unidentified	0.34	0.22	0.34	0.70	0.27	0.50	0.33	0.25
Melamine	0.38	0.51	0.47	0.16	0.22	0.15	0.33	0.32
Metabolite mixture[a]	0.38	0.50	0.92	0.21	0.20	0.13	0.43	0.24
Unidentified	0.13	0.10	IS	IS	0.12	IS	0.50	0.40
Unidentified	IS	IS	IS	IS	IS	IS	0.09	0.14
Cyromazine	3.77	4.84	2.78	2.59	2.26	1.83	5.76	4.95
Unidentified	0.14	0.19	0.04	0.06	0.12	0.07	0.07	0.05
Unidentified	0.04	0.08	< 0.01	0.04	0.11	0.02	< 0.01	< 0.01

From Capps (1990)

IS, Metabolite zones incompletely separated for samples from rats at the lowest dose, compared with those at the highest dose.

[a] Mixture of hydroxy-cyromazine, methyl-cyromazine and minor metabolites.

The predominant metabolic pathway for cyromazine in the rat involves biotransformation to hydroxy-cyromazine and methyl-cyromazine and then to melamine as illustrated in Figure 2.

In male and females rats given a single oral dose of [U-^{14}C triazine]cyromazine at 3 mg or 300 mg/kg bw, the absorbed dose was incompletely metabolized, with cyromazine being the predominant component of both urine and faeces. Cyromazine was metabolized to methyl-cyromazine, hydroxy-cyromazine and melamine. There were no pronounced differences between the sexes and relatively minor differences between the doses. The dosing of rats with unlabelled cyromazine at a dose of 3 mg/kg bw per day for 14 days before a single radiolabelled dose at 3 mg/kg bw had no marked effect on its biotransformation (Capps, 1990).

Monkeys

In a study performed to determine the excretion pattern of cyromazine in monkeys, metabolism was also investigated. Two male and two female *Macaca fasicicula* monkeys were given a single oral dose of [U-^{14}C triazine]-cyromazine at 0.05 mg/kg bw. An additional two male and two female monkeys were given a single oral dose of [U-^{14}C triazine]cyromazine at 0.5 mg/kg bw. The doses were administered in capsules. Each monkey was housed in a metabolism cage to which it had previously been acclimatized. Urine and faeces were collected for 24 h before dosing and at each day after dosing for 4 days. Radioactivity in urine and in combusted samples of faeces was measured by LSC. Aliquots of urine collected over the first 24 h after dosing were analysed by TLC against cyromazine and melamine reference standards. The study was not performed according to GLP, but it was an investigative study designed to complement the regulatory submission on metabolism. The study was regarded as additional information for the evaluation.

In all day-1 urine samples, more than 93% of the radioactivity present was characterized as cyromazine and the remainder as melamine (Table 14). There was considered to be no difference in metabolism between the doses or between the sexes.

In male and female monkeys given a single oral dose of [U-^{14}C triazine]cyromazine at 0.05 or 0.5 mg/kg bw, cyromazine accounted for more than 94% of urinary radioactivity, with the remainder attributable mainly to melamine (Staley, 1986).

Table 14. Relative proportions of cyromazine and melamine in samples of urine collected from monkeys over 24 h after a single oral dose of mg [U-^{14}C triazine]cyromazine at 0.05 or 0.5/kg bw

Sex	Dose (mg/kg bw)	Cyromazine (%)	Melamine (%)
Male	0.05	95.35	3.26
Female	0.05	94.10	5.90
Male	0.5	94.84	3.92
Female	0.5	94.90	3.37

From Staley (1986)

A second study was performed with one male and one female of the same strain of monkey and the same doses given by capsule as in Staley (1986). Each monkey was housed in a metabolism cage to which it had been first acclimatized. Urine and faeces were collected for 24 h before dosing and each day after dosing for 2 days. Radioactivity in urine and in combusted samples of faeces was measured by LSC. Aliquots of urine collected over the first 24 h after dosing were analysed by TLC against cyromazine and melamine reference standards. The study was not performed according to GLP, but it was an investigative study designed to complement the regulatory submission on metabolism. The study was regarded as additional information for the evaluation.

In all day-1 urine samples, approximately 95% or more of the radioactivity present was characterized as cyromazine and the remainder as melamine (Table 15). There was considered to be no difference in metabolism between the doses or between the sexes.

In male and female monkeys given a single oral dose of [U-^{14}C triazine]cyromazine at 0.05 or 0.5 mg/kg bw, cyromazine accounted for approximately 95% or more of urinary radioactivity with the remainder mainly attributable to melamine (Staley & Simoneaux, 1986).

Table 15. Relative proportions of cyromazine and melamine in samples of urine collected from monkeys over 24 h after a single oral dose of [U-^{14}C triazine]cyromazine at 0.05 or 0.5 mg/kg bw

Sex	Dose (mg/kg bw)	Cyromazine (%)	Melamine (%)
Male	0.05	100.00	0.00
Female	0.05	96.08	3.92
Male	0.5	94.99	3.73
Female	0.5	97.01	3.00

From Staley & Simoneaux (1986)

Goats

Lactating goats received [U-^{14}C triazine]cyromazine at a dose equivalent to a dietary concentration of 4.6 and 48.4 ppm for 10 days.

Cyromazine accounted for 43.7%, 35.9%, 0.2% and 32.5% of the extractable radioactivity in the urine, faeces, liver and milk, respectively at the lowest dose and 78.8%, 58.7%, 1.9% and 41.0% at the highest dose, respectively. Melamine concentrations in the urine, faeces, liver and milk were 11.9%, 14.3%, 1.7% and 9.2% at the lowest dose and 7.8%, 10.4%, 5.6% and 4.5% at the highest dose, respectively. 1-Methylcyromazine accounted for 44.4%, 17.4%, 92.7% and 1.0% of the extractable radioactivity at the lowest dose and 13.4%, 1.8%, 71.7% and 0.2% at the highest dose in urine, faeces, liver and milk, respectively (Simoneaux & Marco, 1984). This study had been evaluated by the 1990 JMPR and was not reviewed by the present Meeting.

Hens

Two hens received capsules containing [U-^{14}C triazine]cyromazine at a dose equivalent to a dietary concentration of 5.0 ppm for 7 days.

Most of the excreted radioactivity (75%) was unaltered cyromazine. A metabolite, incompletely characterized in this study but with the same retention characteristics as melamine, was found in egg white at 0.04 mg/kg and egg yolk at 0.01 mg/kg when the hens were fed diet containing cyromazine at 5 mg/kg.

The metabolism of cyromazine in hens appears to proceed by the same major pathway as in goat and rat, namely via dealkylation of the cyclopropyl group to yield the tri-amino-*s*-triazine, melamine (Simoneaux & Cassidy, 1979). This study had been evaluated by the 1990 JMPR and was not reviewed by the present Meeting.

Cow

In a feeding study in cows, 1-methylcyromazine was found at low levels of up to 0.11 ppm in the liver. It was below the level of quantification (LOQ) in milk, muscle, kidney, blood and fat. This metabolite was also found at a level of about 2% in urine of rats.

1.3 Dermal absorption

The dermal absorption of [U-^{14}C triazine]cyromazine (specific activity, 2590 kBq/mg, for the highest dose; the labelled test substance was diluted with non-labelled cyromazine to a specific activity of 70 kBq/mg; radiochemical purity, 98%), formulated as a wettable powder (WP) containing 7.5% active substance, was tested in groups of 16 rats at doses of 1.0 (lowest dose, P1) and 20 µg cyromazine/cm^2 (intermediate dose, P2), reflecting the range of typical concentrations recommended for the use in the field and 750 µg cyromazine/cm^2 (highest dose, P3) which represents the concentrate formulation (active substance, 75 g/l). Actual applied doses as cyromazine were 1.2, 23 and 864 µg/cm^2. The test substance was applied for 6 h to a shaved dorsal area of about 10 cm^2 of male rats (HanBrl: WIST (SPF). The application area was restricted by a non-absorbing 'O'-ring glued to the clipped skin using a cyanoacrylate adhesive. The application volume was 100 µl per animal. In order to prevent loss of the test substance, the 'O'-ring was covered with a non-occlusive cover tape. During the experiment, the rats were housed in all-glass metabolism cages. The depletion of the amount remaining in/on the skin after washing was determined on subgroups of four animals at four time-points, i.e. 6, 24, 48 and 72 h after application. The location within the skin of the skin-associated radioactivity was determined by removing the stratum corneum from the treated skin by tape stripping. After the 6-h exposure period, the cover was removed and retained for analysis. The unabsorbed material was removed from the application site by washing five times with a mild soap solution using cotton swabs. After each washing step, the skin was dried with a cotton swab. Finally a fresh cover was applied to the 'O'-ring. At necropsy, the 'O'-ring and the cover were carefully removed and extracted with methanol. Urine and faeces were collected from individual animals at 0–6, 6–24, 24–48, and 48–72 h after application. Blood was taken from the tail vein from each animal in the subgroup in which depletion was measured until 72 h after application at 0.5, 1, 2, 4, 6, 8, 24, and 48 h after application. In order to follow the fate of the applied test substance more closely, the treated skin was fractionated by tape-stripping into a fraction representing the stratum corneum and the remaining treated skin (epidermis and dermis). Radioactivity was determined in blood samples, urine, faeces, cage wash, skin wash, shavings, tape-strippings, residual skin of the application site, control skin, wash solution of 'O'-ring/cover and residual carcass. Additionally, TLC analysis of the dosing solution and of the skin wash was performed. The animals were checked for appearance and behaviour during acclimatization and at each sampling time-point. Some animals showed slight stress symptoms, i.e. chromodacryorrhoea and encrustations around the nostrils, during the first hour after administration. Also the slight weight loss of the animals was attributed to the

stress and discomfort during the experiment, e.g. the collar around the neck, the glued 'O'-ring on the dorsal area and the bandage. The study was conducted according to the principles and practices of GLP (with QA certificate) and the protocol was in accordance with OECD TG 427 (2000).

The test substance was stable in the formulation, as indicated by the radiochemical purity of > 98%. TLC analysis of the skin wash solution revealed that more than 96% of the radioactivity was unchanged cyromazine.

The experimental recoveries were determined to be between 90% and 98% of the applied dose (subgroup mean). After an exposure time of 6 h, 3.20%, 1.93%, 2.04%, and 1.54% of the applied cyromazine were systemically absorbed within 6 h, 24 h, 48 h, and 72 h after application, indicating a moderate absorption of cyromazine through rat skin at the lowest dose (Table 16).

Table 16. Dermal absorption of [U-^{14}C triazine]cyromazine at an applied dose of 1.2 µg/cm² in rats[a]

Sample	Mean percentage of applied dose			
	Sacrifice time			
	6 h	24 h	48 h	72 h
Urine				
0–6 h	2.60	1.40	1.41	0.78
6–24 h	—	0.38	0.40	0.35
24–48 h	—	—	0.10	0.16
48–72 h	—	—	—	0.07
Subtotal	2.60	1.78	1.90	1.35
Faeces				
0–6 h	0.04	0.01	0.04	0.04
6–24 h	—	0.03	0.01	0.01
24–48 h	—	—	0.03	0.02
48–72 h	—	—	—	0.04
Subtotal	0.04	0.04	0.09	0.12
Cage wash	0.13	0.06	0.03	0.04
Total excretion	2.76	1.87	2.02	1.51
Residues				
Whole blood[b]	0.07	0.03	0.02	< 0.01
Skin non-treated[b]	< 0.01	< 0.01	< 0.01	< 0.01
Gastrointestinal tract	< 0.01	0.03	< 0.01	0.03
Remaining carcass	0.37	< 0.01	< 0.01	< 0.01
Subtotal	0.43	0.06	0.02	0.04
Systemic absorption	3.20	1.93	2.04	1.54
Skin stripping	11.25	16.54	16.62	17.70
Remaining treated skin	0.25	0.63	0.12	0.20
Application site	11.50	17.17	16.74	17.90
Skin wash	65.21	65.28	68.32	69.40
Cover and 'O'-ring	15.63	11.15	11.12	6.90
Dislodged dose	80.83	76.43	79.44	76.30
Total recovery	95.53	95.54	98.22	95.74

From Hassler (2002a)

[a] Corresponds to group P1.

[b] Residues determined in the portion of the specimen.

At the intermediate dose, the relative dermal penetration of cyromazine accounted for 2.14%, 1.71%, 2.51%, and 2.06% of the applied dose at 6, 24, 48, and 72 h (Table 17).

At the highest dose the relative dermal penetration of cyromazine accounted for 0.99, 0.97, 0.58, and 0.37% of the applied dose at 6, 24, 48, and 72 h (Table 18).

Table 17. Dermal absorption of [U-^{14}C triazine]cyromazine at an applied dose of 23 µg/cm^2 in rats[a]

Sample	Mean percentage of applied dose			
	Sacrifice time			
	(6 h)	(24 h)	(48 h)	(72 h)
Urine				
0–6 h	1.81	1.12	1.62	1.53
6–24 h	—	0.40	0.69	0.30
24–48 h	—	—	0.12	0.10
48–72 h	—	—	—	0.04
Subtotal	1.81	1.51	2.43	1.96
Faeces				
0–6 h	0.05	0.02	0.01	0.03
6–24 h	—	0.01	0.03	0.01
24–48 h	—	—	< 0.01	< 0.01
48–72 h	—	—	—	< 0.01
Subtotal	0.05	0.03	0.05	0.05
Cage wash	0.07	0.02	0.03	0.02
Total excretion	1.93	1.56	2.51	2.03
Residues				
Whole blood[b]	< 0.01	< 0.01	< 0.01	< 0.01
Skin, non-treated[b]	< 0.01	< 0.01	< 0.01	< 0.01
Gastrointestinal tract	0.02	< 0.01	< 0.01	< 0.01
Remaining carcass	0.18	0.15	< 0.01	0.02
Subtotal	0.21	0.15	< 0.01	0.03
Systemic absorption	2.14	1.71	2.51	2.06
Skin-stripping	12.01	13.68	12.88	15.53
Remaining treated skin	0.23	1.09	0.59	0.60
Application site	12.24	14.77	13.47	16.13
Skin wash	67.16	64.23	66.39	76.72
Cover and 'O'-ring	14.66	14.06	13.10	0.78
Dislodged dose	81.82	78.28	79.49	77.50
Total recovery	96.20	94.77	95.47	95.70

From Hassler (2002a)

[a] Corresponds to group P2

[b] Residues determined in the portion of the specimen.

Table 18. Dermal absorption of [U-^{14}C triazine]cyromazine at an applied dose of 864 µg/cm² in rats[a]

Sample	Mean percentage of applied dose			
	Sacrifice time			
	6 h	24 h	48 h	72 h
Urine				
0–6 h	0.72	0.79	0.42	0.21
6–24 h	—	0.12	0.10	0.08
24–48 h	—	—	0.02	0.03
48–72 h	—	—	—	0.01
Subtotal	0.72	0.91	0.54	0.34
Faeces				
0–6 h	< 0.01	0.02	< 0.01	< 0.01
6–24 h	—	< 0.01	< 0.01	< 0.01
24–48 h	—	—	< 0.01	< 0.01
48–72 h	—	—	—	< 0.01
Subtotal	< 0.01	0.02	< 0.01	0.02
Cage wash	0.23	0.02	< 0.01	0.01
Total excretion	0.95	0.95	0.56	0.36
Residues				
Whole blood[b]	< 0.01	< 0.01	< 0.01	< 0.01
Skin non-treated[b]	< 0.01	< 0.01	< 0.01	< 0.01
Gastrointestinal tract	< 0.01	< 0.01	< 0.01	< 0.01
Remaining carcass	0.04	0.02	0.02	< 0.01
Subtotal	0.04	0.02	0.03	< 0.01
Systemic absorption	0.99	0.97	0.58	0.37
Skin stripping	1.44	2.36	2.40	3.35
Remaining treated skin	0.07	0.32	0.02	0.02
Application site	1.51	2.68	2.43	3.37
Skin wash	82.96	86.28	84.86	85.98
Cover and 'O'-ring	5.10	0.45	2.13	1.08
Dislodged dose	88.06	86.73	86.99	87.06
Total recovery	90.57	90.38	90.00	90.79

From Hassler (2002a)

[a] Corresponds to group P3.

[b] Residues determined in the portion of the specimen.

The penetration rates during the 6-h exposure time, calculated from results of 6 h subgroups were 0.0064 µg cm^{-2} h^{-1} for the lowest dose (group P1), 0.0810 µg cm^{-2} h^{-1} for the intermediate dose (group P2), and 1.4326 µg cm^{-2} h^{-1} for the highest dose (group P3).

Because of the very low extent of absorption, the blood residues during and after dermal exposure at all doses were at or below the limit of determination.

About 76–81%, 77–82%, and 87–88% of the applied dose could be dislodged from the application site at the end of the exposure period at the lowest, intermediate and highest dose, respectively. The radioactivity that remained associated with the treated skin accounted for 11.5%, 12.2% and 1.5% of the lowest, intermediate and highest dose, respectively, at 6 h after application of the test substance and just after the skin-wash.

In order to follow the fate of cyromazine in the skin more closely, the skin was fractionated by tape-stripping the stratum corneum. At all doses the radioactivity remained in/on the treated skin area and was associated with the stratum corneum, i.e. 11–18% of the lowest dose, 12–16% of the intermediate dose, and 1–3% of the highest dose. The lower skin layers after skin-stripping, i.e. corium and subcutis, showed insignificant concentrations of radioactivity, indicating that the radioactivity present in the stratum corneum was not available for further penetration into the lower skin layers. The systemically absorbed dose was rapidly eliminated mainly via the urine. Elimination via the faeces was only a minor route of excretion (Hassler, 2002a).

Penetration of [U-^{14}C triazine]cyromazine (specific activity, 2590 kBq/mg, for the highest dose, the labelled test substance was diluted with non-labelled cyromazine to a specific activity of 70 kBq/mg; radiochemical purity, 98%), formulated as a WP containing 7.5% active substance, through rat and human epidermis was compared in vitro. The epidermal membranes were mounted to flow-through cells and each diffusion cell received a 6 µl aliquot of the application solution. Cyromazine was applied at a concentration of 1.1, 22 or 834 µg/cm^2 to rat or human epidermis for 6 h (Table 19). The two lowest doses reflected typical concentrations recommended for the use in the field, while the highest dose represented the concentrate formulation (active ingredient, 75 g/l).

Table 19. Dermal penetration of cyromazine through rat and human epidermis in vitro

Species	Dose	Applied dose		Concentration	
		mg/cell[a]	mg·cm^{-2}[a]	KBq/cell	(mg·cm^{-3})[a]
Rat	Lowest	0.7	1.1	1.9	0.12
	Intermediate	14	22	37	2.4
	Highest	534	834	37	89
Human	Lowest	0.7	1.1	1.9	0.12
	Intermediate	14	22	37	2.4
	Highest	534	834	37	89

[a] Expressed as cyromazine

From Hassler (2002b)

Rat epidermis was prepared from male HanBrl:WIST (SPF) rats aged about 9 weeks. Human epidermis was prepared from abdominal cadaver skin from Caucasian donors. Perfusates were collected at defined time-points. Twenty-four hours after application, the skin membrane surface was rinsed with ethanol (10 ml) and the radioactivity in the skin rinse was determined by LSC. The skin membrane was removed from the in-line cells and dissolved in tissue solubilizer before LSC. The receptor chamber was washed with ethanol and radioactivity determined by LSC. The study was conducted according to the principles and practices of GLP (with QA certificate) and the protocol was in accordance with OECD TG 428 (2000).

A TLC check for test substance stability of the formulated material revealed that [U-^{14}C triazine] cyromazine represented more than 98% of the radioactivity. The permeability check of the membranes revealed mean permeability constants (Kp) of tritiated water in the range of 0.54–0.98 × 10^{-3} cm/h and 0.53–0.96 × 10^{-3} cm/h for rat and human epidermis, respectively. At the end of the experiment, the skin rinse was analysed. In both groups, unchanged cyromazine amounted to more than 97% of the radioactivity present in the skin rinse. It was concluded that cyromazine remained unchanged for 24 h on the epidermis.

Within 24 h, 16.9% of the lowest dose, 19.1% of the intermediate dose, and 0.25% of the highest dose penetrated the rat epidermis membrane (Table 20), corresponding to penetration of 0.19 µg·cm^{-2}, 4.21 µg·cm^{-2}, and 2.07 µg·cm^{-2}, respectively. The flux, which reflects the penetration

rate under steady-state conditions, amounted to 0.018 $\mu g \cdot cm^{-2} \cdot h^{-1}$, 0.560 $\mu g \cdot cm^{-2} \cdot h^{-1}$ and 0.370 $\mu g \cdot cm^{-2} \cdot h^{-1}$ at the lowest, intermediate and highest dose, respectively. While the concentration for the highest dose was 700-fold that at the lowest dose, the penetration rate at the highest dose was only 20-fold that at the lowest dose. Comparing the penetration rates of the highest dose with the intermediate dose, the calculated penetration rate remained almost constant while the concentration of the test substance at the highest dose was 40-times that at the intermediate dose. This observation was attributed to the limited solubility of the test substance in aqueous solution, i.e. 13 $mg \cdot cm^{-3}$. While the test substance at the lowest and intermediate dose formulations was dissolved, the concentration at the highest dose significantly exceeded the solubility of the test substance.

Within 24 h, 2.3% of the lowest dose, 1.1% of the intermediate dose, and 0.05% of the highest dose penetrated through the human skin membrane, corresponding to a penetration of 0.03 $\mu g \cdot cm^{-2}$, 0.25 $\mu g \cdot cm^{-2}$, and 0.40 $\mu g \cdot cm^{-2}$, respectively. The calculated flux, under steady-state conditions, accounted for 0.002 $\mu g \cdot cm^{-2} \cdot h^{-1}$, 0.021 $\mu g \cdot cm^{-2} \cdot h^{-1}$, and 0.064 $\mu g \cdot cm^{-2} \cdot h^{-1}$ at the lowest, intermediate, and highest dose, respectively. However, based on the low specific activity and the very low penetration rate at the highest dose, most of the determined values were at or below the limit of determination. Therefore this calculated penetration rate (flux) was considered to be not very trustworthy. Again, the 700-times higher concentration of the highest dose led only to a 30-times higher penetration rate (flux) when compared with the lowest dose.

At the end of the experiments, the recovered radioactivity in the perfusate, skin rinse, skin membrane, and cell wash was measured (Table 21). Total recovery of radioactivity ranged from 98% to 103% and 96% to 103% of the applied radioactivity for rat and human skin membranes, respectively. Cyromazine amounted to more than 97% of the radioactivity present in the skin rinses for all doses and both species. Hence, it was concluded that the test substance remained essentially unchanged during the 24 h of exposure on the skin membrane.

Table 20. Dermal penetration of cyromazine through rat and human epidermis in vitro

Penetration	Applied dose ($\mu g \cdot cm^{-2}$)	Concentration ($mg \cdot cm^{-3}$)	Applied dose ($\mu g \cdot cm^{-2}$)	Concentration ($mg \cdot cm^{-3}$)	Applied dose ($\mu g \cdot cm^{-2}$)	Concentration ($mg \cdot cm^{-3}$)
	1.1	0.12	22	2.35	834	88.92
	Lowest dose		Intermediate dose		Highest dose	
	Percentage of dose	$\mu g \cdot cm^{-2}$	Percentage of dose	$\mu g \cdot cm^{-2}$	Percentage of dose	$\mu g \cdot cm^{-2}$
Rat epidermis						
Penetration at:						
6 h	6.26	0.07	7.93	1.75	0.16	1.37
12 h	10.64	0.12	12.07	2.66	0.20	1.68
24 h	16.85	0.19	19.09	4.21	0.25	2.07
Flux ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	0.018		0.560		0.370	
Human epidermis						
Penetration at:						
6 h	0.47	0.01	0.28	0.06	0.02	0.15
12 h	0.87	0.01	0.54	0.12	0.03	0.24
24 h	2.27	0.03	1.14	0.25	0.05	0.40
Flux ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	0.002		0.021		0.064	

From Hassler (2002b)

Table 21. Recovery of radioactivity in a study of dermal penetration with cyromazine in rat and human epidermis in vitro

Sample	Recovery (percentage of administered dose)					
	Rat epidermis			Human epidermis		
	Dose (µg·cm^{-2})					
	Lowest dose	Intermediate dose	Highest dose	Lowest dose	Intermediate dose	Highest dose
	1.1	22	834	1.1	22	834
Perfusates:						
0–24 h	16.85	19.09	0.25	2.27	1.14	0.05
Remaining dose:						
Cell wash	2.90	3.23	2.22	2.69	0.33	2.77
Skin rinse	35.47	59.47	71.43	62.57	91.53	84.09
Skin membrane	48.13	17.65	24.12	35.54	6.71	9.39
Subtotal	86.49	80.35	97.77	100.80	98.57	96.24
Total recovery	103.35	99.44	98.02	103.08	99.71	96.29

From Hassler (2002b)

Cyromazine, (formulated as a WP containing 7.5% active substance) penetrated at a faster rate and to a greater extent through rat than human split-thickness skin membranes, at all doses tested. The species difference was reflected in the flux constants determined as 0.018, 0.560 and 0.370 µg·cm^{-2}·h^{-1} through rat epidermis and 0.002, 0.021 and 0.064 µg·cm^{-2}·h^{-1} through human epidermis at the lowest, intermediate and highest dose, respectively. The resulting rat : human ratio of the flux constants was about 9 : 1, 27 : 1 and 6 : 1 at the lowest, intermediate and highest dose, respectively (Hassler, 2002b).

On the basis of the results of the dermal absorption study in rats in vivo and the comparative skin penetration study through rat and human skin in vitro, dermal penetration values for humans in vivo can be calculated by dividing the dermal absorption obtained in the rat in vivo by the ratio of the absorption rates between rat and human in vitro. In humans, the dermal absorption of cyromazine, formulated as a WP containing 7.5% active substance, was 0.1% for the concentrate and 0.1% for the highest and 0.3% for the lowest spray concentrations used in the field (Table 22).

Table 22. Calculation of dermal absorption of cyromazine[a] in humans

Parameter	Lowest dose 0.0011 mg/cm² (0.12 g/l)	Intermediate dose 0.022 mg/cm² (2.4 g/l)	Highest dose 0.834 mg/cm² (89 g/l)
Absorption in rats after 6 h (% of dose)	3.20	2.14	0.99
Absorption in rats after 72 h (% of dose)	1.54	2.06	0.37
Flux in rats (µg·cm-2·h^{-1})	0.018	0.560	0.370
Flux in humans (µg·cm-2·h^{-1})	0.002	0.021	0.064
Flux ratio (rat/human)	9	27	6
Absorption in humans (% of dose)	0.3	0.1	0.1

[a] Formulated as a wettable powder containing 7.5% active substance.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The acute toxicity of cyromazine after administration by the oral, dermal and inhalation routes is summarized in Table 23.

Table 23. Results of studies of acute toxicity with cyromazine

Species	Strain	Sex	Vehicle	LD_{50} (mg/kg bw; 95% CI or range) LC_{50} (mg/l air)	GLP or QA	Reference
Oral administration						
Rats	Tif:RAIf (SPF)	M & F	PEG 400	3387 (2524–4547)[a]	—	Sachsse & Bathe (1978a)
Inhalation						
Rats	HSD:SD	M & F	Aerosol, 4-h, nose-only	> 3.6 (maximum attainable concentration)[b]	GLP & QA	Holbert (1994)
Dermal administration						
Rats	Tif:RAIf (SPF)	M & F	PEG 400	> 3170[c]	—	Sachsse & Bathe (1978b)

F, female; GLP, good laboratory practice; M, male; PEG 400, polyethylene glycol; QA, quality assurance.

[a] Groups of five male and five female fasted rats received cyromazine (unknown purity) as a single gavage dose at 1000, 1670, 3590, 4640 or 6000 mg/kg bw as a suspension in PEG 400. The animals were observed for 14 days before sacrifice. Animals found dead and those surviving until the end of the study were subjected to macroscopic examination post mortem. The study was performed before the implementation of GLP and before EU or OECD guidelines had been enacted, but essentially conformed to OECD TG 401 (1987). Within 2 h of dosing, rats at all doses showed sedation, dyspnoea, exophthalmos, curved position and ruffled fur. Mortality occurred in zero, five (four on day 1 and one on day 7), two (day 1) and three (two on day 1 and one on day 2) males and in one (day 2), one (day 2), five (four on day 1 and one on day 2) and five (four on day 1 and one on day 2) females at 1000, 1670, 3590, 4640 and 6000 mg/kg bw, respectively. The surviving animals recovered within 9 to 12 days. There were no treatment-related macroscopic findings.

[b] Two groups of five male and five female rats were exposed nose-only for a single 4-h period to an aerosol generated from undiluted cyromazine technical (purity, 96.5%) at a mean concentration of 0.744 or 3.6 mg/l air. A control group of five males and five females was exposed to air only under similar experimental conditions. Clinical signs were recorded twice on the day of exposure and at least daily thereafter for up to 17 days. Body weights were measured before exposure, on day 7 and at the day of termination. At termination of the study, all animals were subjected to gross necropsy.

A maximum attainable exposure concentration of 3.6 mg/l was obtained, with a mean mass median aerodynamic diameter of 6.68 µm (3.92 µm at 0.744 mg/l). The study was performed according to Pesticide Assessment Guidelines, subdivision F, hazard Evaluation: Human and Domestic Animals, Series 81-3, EPA Publication, EPA 540/9-84-014, November 1984 and is in compliance with TM B2 of Dir. 92/69/EEC

None of the animals died before the scheduled termination. Prominent in-life observations included activity decrease, piloerection at both concentrations and additionally nasal discharge at the higher concentration. Clinical signs were no longer seen on day 2. Body-weight gain was largely unaffected. Two females at 3.6 mg/l and two females in the control group lost weight between days 0 and 7. Gross necropsy revealed discolouration of the lungs in animals of all groups and multiple red foci on lungs of males at 0.744 mg/l.

[c] Groups of five male and five female rats received a single 24-h dermal application of cyromazine (unknown purity) at 2150 or 3170 mg kg bw as a suspension in PEG 400. The animals were assessed for 14 days for any signs of systemic toxicity or skin irritation. At the end of the observation period all animals were killed and examined macroscopically. The study was performed before the implementation of GLP and before EU or OECD guidelines had been enacted, but essentially conformed to OECD TG 402 (1987).

None of the animals died. Within 24 h of treatment the rats in both groups showed dyspnoea, curved position and ruffled fur. No local skin irritation was seen. The animals recovered from systemic symptoms within 10 days. There were no treatment-related macroscopic findings.

Cyromazine is of low acute toxicity via the oral, dermal and inhalation routes. In rats, the oral median lethal dose (LD_{50}) for males and females combined was 3387 mg/kg bw; the dermal LD_{50} was greater than 3170 mg/kg bw and the inhalation LC_{50} (4-h exposure) was greater than 3.6 mg/l air, the highest achievable concentration. Symptoms of intoxication were sedation, dyspnoea, curved position and ruffled fur after oral or dermal administration. Animals recovered from systemic symptoms within 9–12 days. After inhalation, activity decrease, piloerection and nasal discharge were observed; these clinical signs were no longer seen on day 2.

(b) Dermal and ocular irritation and dermal sensitization

(i) Dermal irritation

A group of three male and three female Himalayan rabbits received a single 24-h occlusive application of approximately 0.5 g of cyromazine (unknown purity) to an abraded or non-abraded area of the shorn flank. The animals were assessed for any signs of skin irritation immediately and 48 h after removal of the dressings (24 h and 72 h after initiation of treatment). The Draize scale was used to assess the degree of erythema and oedema at the application sites. Mean erythema and oedema scores were calculated. The study was performed before the implementation of GLP and before EU or OECD guidelines had been enacted. Deviations to OECD TG 404 (1987) are considered not to compromise the scientific validity of the study.

On intact skin, very slight erythema (score 1) was noted in two animals at 24 h and very slight oedema (score 1) was recorded in four animals. All signs of skin irritation had disappeared 72 h after initiation of treatment. Mean scores for erythema and oedema were 0.17 and 0.3, respectively. On the basis of the these findings, cyromazine was not considered to be a skin irritant (Sachsse & Ullmann, 1978a).

(ii) Ocular irritation

Cyromazine (unknown purity) was not an irritant to the eye of Himalayan rabbits when assessed essentially in compliance with OECD TG 405 (1987) in a study that was performed before the implementation of GLP (Sachsse & Ullman, 1978b).

(iii) Dermal sensitization

The sensitization potential of technical cyromazine (purity, 97.4%) was assessed according to the maximization test of Magnusson & Kligman (1969). Groups of 10 male and 10 female young Himalayan Spotted (GOHI) guinea-pigs (test) and a control group of five males and five females were used for the main study. In test animals, the induction phase involved three intradermal injections of: (a) a 5% w/v preparation of cyromazine in the vehicle (0.5% carboxymethylcellulose sodium salt (CMC) and 0.1% Tween 80 in doubly-distilled water); (b) a 5% w/v preparation of cyromazine in a 1 : 1 preparation of saline : Freund's complete adjuvant (FCA); and (c) a 1 : 1 preparation of saline : FCA to a shorn area of the scapular region on day 1. This was followed 1 week later by a topical induction using the test substance (75% in 0.5% CMC and 0.1% Tween 80 in doubly-distilled water) under an occlusive dressing for 48 h. For guinea-pigs in the control group, the intradermal injections were saline : FCA and vehicle (0.5% CMC and 0.1% Tween 80 in doubly-distilled water) alone, and the topical applications were as for the test animals except that vehicle only was applied. Application sites were checked 24 h and 48 h after removal of the dressings. In the challenge phase, 2 weeks after completion of the induction phase, 50% cyromazine was applied to the shorn left flank and vehicle only was applied to the shorn right flank of test animals under an occlusive dressing for 24 h. Skin sites were examined approximately 2 h and 48 h after removal of the dressings. A study with a positive control was conducted using essentially the same methodology and using 2-mercaptobenzothiazole as

the test substance. The method used an intradermal induction of a 5% w/v preparation in mineral oil and in an emulsion of FCA : saline, 15% w/v preparation in mineral oil for the topical induction phase and 1% w/v preparation in mineral oil for the challenge phase. The study was conducted in compliance with the principles of GLP (with QA certificate) and according to OECD TG 406 (1992).

There were no skin reactions among the guinea-pigs in the control group. There were no positive skin reactions on the test flanks or control flanks of the test-group animals, corresponding to a sensitization rate of 0%. Therefore, cyromazine is not a skin sensitizer (Arcelin, 2000).

2.2 Short-term studies of toxicity

Rats

Groups of 20 male and 20 female Charles River CD rats were fed diets containing cyromazine (purity, 96.3%) at a concentration of 0, 30, 300, 1000 or 3000 ppm for up to 90 days. The doses were equal to a mean daily intake of 0, 2.4, 23, 79 and 232 mg/kg bw per day in males and 0, 2.6, 27, 88 and 264 mg/kg bw per day in females. An additional five rats of each sex per group were assigned to the control group and the group at 3000 ppm; they were kept for 4 weeks after the end of dosing to study recovery. Mortality and clinical signs were checked twice per day, body weights and food consumption were recorded weekly. Ophthalmological examinations were conducted in all animals before dosing, in week 13 and for recovery rats in week 16. Samples for laboratory investigations (haematology, clinical chemistry and urine analysis) were taken from 10 rats per dose and sex at intervals during the study (day 31, 58 and 86 for blood and day 31, 58, 83 and 89 for urine) and from recovery animals before termination. All animals were necropsied at terminal sacrifice, selected organs were weighed and selected organs and tissues were examined histopathologically. The study was performed before GLP had been formally adopted and before OECD TG 408 (1981) had been enacted, but complied to a great extent with these requirements.

There was no mortality and no treatment-related signs of toxicity were observed. Cyromazine triggered a slightly reduced body-weight gain at 1000 and 3000 ppm (reduction compared with controls of 5% and 8% in males and 7% and 8% in females, respectively). Body weight recovered during the 4-week recovery period. Food consumption remained unaffected. Ophthalmic examinations, haematological and biochemical tests and urine analyses revealed no compound-related effects.

There were no macroscopic or microscopic findings post mortem that could be attributed to treatment with cyromazine. Statistically significant differences in the relative liver weights were scattered over all dose groups. However, in the absence of any effects in clinical chemistry, macropathological or histopathological findings, these observations were considered to be of no biological relevance.

The NOAEL was 3000 ppm, equal to 232 mg/kg bw per day in males and 264 mg/kg bw per day in females, the highest dose tested considering that the slight reductions of body-weight gain observed at 1000 and 3000 ppm were not toxicologically relevant (Goldenthal, 1979).

In a study that complied with the principles of GLP (with QA certificate provided), groups of five male and five female Tif:RAIf (SPF) rats were exposed nose-only to cyromazine (purity not stated) in an aerosol at a concentration of 0, 0.058, 0.206 or 0.706 mg/l air for 4 h per day during 28 consecutive days. Additional groups of five males and five females treated at 0 and 0.706 mg/l air were maintained for a 3-week recovery period. Rats in the control group were exposed to filtered humidified air. Mortality and clinical signs were checked at least daily, body weights and food consumption were recorded weekly. Samples for laboratory investigations (haematology and blood clinical chemistry) were taken from all rats at the end of the treatment period, and from recovery animals after a 2-week treatment-free period. All animals were necropsied at terminal sacrifice,

selected organs were weighed, and selected organs and tissues were examined histopathologically. The study complied to a great extent with OECD TG 412 (1981), except that exposure was 4 h per day instead of 6 h per day.

There was no mortality. Treatment with cyromazine triggered clinical signs that included piloerection, dyspnoea, hunched posture, and reduced spontaneous activity in all treated groups. The time of onset and severity was concentration-dependent and subsided during the recovery period. There were no statistically significant, dose-related effects on body weights in either males or females; however, males in all exposure groups showed a slightly depressed body weight compared with controls at the end of the exposure period. The depression was independent of the dose and showed recovery by the end of the recovery period. There were no significant exposure-related effects on food consumption.

Minor effects noted in haematology were slightly higher values for erythrocyte parameters, specifically erythrocyte count, haemoglobin and erythrocyte volume fraction, in the males at the highest dose. At the end of the recovery period, the values for treated and control groups were comparable. There was no evidence that treatment with cyromazine had any influence on blood clinical chemistry parameters. Incidences of statistically significant differences between treated groups and control were sporadic and not dose-related.

Organ-weight analysis showed decreased pituitary weights in all treated males and increased liver weights at doses of 0.206 and 0.706 mg/l in treated females. However, pituitary weight in treated females was higher than that of controls, but did not reach statistical significance. In view of the small size of the pituitary and therefore the high variability in the weight, and in the absence of histopathological findings in this organ, the biological significance of this result is questionable. Pathology was essentially unremarkable. Microscopic examination revealed three females from the group at 0.706 mg/l and one female from the group at 0.058 mg/l with small foci of lymphocytic infiltration in the adrenal cortex. This change was not seen in females in the control group. All other changes were incidental in nature and not related to the test compound.

Daily exposure to cyromazine for 28 days resulted in slight, non-specific toxicity in rats. At the lowest concentration of 0.058 mg/l, equivalent to 9.3 mg/kg bw per day,[1] cyromazine triggered marginal effects in the rats' general condition only. In the absence of a substantial impairment of body-weight development, haematological and pathological parameters and clinical signs of only slight degree, is the Meeting considered this to be a no-observed-adverse-effect concentration (NOAEC). Exposure at 0.206 mg/l produced moderate signs of toxicity. Exposure at 0.706 mg/l produced moderate to severe signs of toxicity after 1 week of exposure (Hartmann, 1988).

Rabbits

In a study that complied with the principles of GLP (with QA certificate provided), groups of five male and five female New Zealand White rabbits were treated dermally with cyromazine (purity, 94.6%) 50, 500 or 2000 mg/kg bw per day and a vehicle control group received deionized water only. Application to the clipped dorsal surface lasted for 6 h per day, 5 days per week during 3 weeks. The test site, approximately 10% of the surface area, was moistened with 3.0 ml of deionized water and covered with a semi-occlusive dressing. One additional male was added to the group at 50 mg/kg bw and two males were added to the group at 500 mg/kg bw due to early non-treatment related deaths. Observations for mortality were made twice per day and the animals were assessed daily for any signs of systemic toxicity. Body weights and food consumption were recorded at intervals throughout the study. Twenty-four hours after the final application, the rabbits were killed and blood samples

[1] Twenty-four hour respiratory volume for rats is 0.096 m^3/kg bw (Zielhuis & Van der Kreek, 1979).

taken for haematological and biochemical analysis. Each rabbit was subjected to a macroscopic examination post mortem, selected organs weighed and selected tissues examined microscopically. The study complied with OECD TG 410 (1981).

Several deaths occurred with no dose–response relationship i.e. there were no deaths at the highest dose, while deaths occurred in the control group (one male), at 50 mg/kg bw (one female) and at 500 mg/kg bw (two males and two females). Clinical observations were generally minor. Changes in bowel and bladder function were observed in all groups and occurred more frequently during the first part of the study; these changes could have been in response to wrapping and handling of the rabbits. Other clinical observations included decreased activity, emaciation, lacrimation, yellow nasal discharge and ataxia; however, these effects were not dose-related and were considered not to be related to treatment. The 21-day dermal exposure to cyromazine produced no observable skin irritation. There were no dose-related effects on body weight or food consumption.

There were no dose-related differences in the clinical chemistry or haematological parameters measured in treated rabbits compared with those in the control group. There were no dose-related differences in organ weights, organ : body weight ratios, organ : brain weight ratios or histopathology in treated rabbits compared with those in the control group. A number of histopathological changes indicative of low-grade infection or parasite migration were observed in liver, kidneys and lungs taken at sacrifice from all four groups. These findings were considered not to be related to treatment.

In the absence of any treatment-related effect, the NOAEL was 2000 mg/kg bw per day, the highest dose tested (Kuhn, 1992).

Dogs

Groups of four male and four female beagle dogs were fed diets containing cyromazine (purity, 96.3%) at a concentration of 0, 30, 300, 1000 or 3000 ppm for up to 90 days. The doses were equal to a mean daily intake of 0, 1.2, 11, 36 and 100 mg/kg bw per day in males and 0, 1.1, 12, 33 and 96 mg/kg bw per day in females. An additional two dogs of each sex per group were assigned to the control group and the group at 3000 ppm; they were maintained for 4 weeks after the end of dosing to study recovery. Mortality and clinical signs were checked daily, body weights and food consumption were recorded weekly. Ophthalmological examinations were conducted in all dogs before dosing, in week 12 and for dogs in the recovery group in week 17. Samples for laboratory investigations (haematology, clinical chemistry and urine analysis) were taken from all dogs at intervals during the study (before dosing and after 4, 8 and 12 weeks) and from recovery animals before termination. All dogs were necropsied at terminal sacrifice, selected organs were weighed and selected organs and tissues were examined histopathologically. The study was performed before GLP had been formally adopted and before OECD TG 409 (1981) had been enacted but complied to a great extent with these requirements.

There was no mortality. Slightly relaxed nictitating membranes were quite frequently observed among treated dogs as was slightly dry nose among dogs at 1000 and 3000 ppm; in control dogs, relaxed nictitating membranes and dry nose were rarely encountered. Other findings in general behaviour, appearance and ophthalmoscopic examinations were similar for dogs in the control and test groups. Cyromazine triggered some reduction in body-weight gain in both sexes at 3000 ppm (reduction of 36% and 58% compared with controls in males and females, respectively) and in females at 1000 ppm (reduction of 38% compared with controls). During the recovery period, dogs (especially males) that had received cyromazine at 3000 ppm showed slightly greater increases in body weight than did dogs in the control group. Food consumption was slightly decreased for dogs at 3000 ppm when compared with dogs in the control group (decrease of 8.5% and 15.3% compared with controls in males and females, respectively). During the recovery period, food consumption for dogs previously treated at 3000 ppm exceeded that of dogs in the control group.

Cyromazine lowered erythrocyte parameters (total erythrocyte, haemoglobin and erythrocyte volume fraction values) in males at 3000 ppm (4–10%). These effects were considered to be physiologically significant but not pathological. They were reversible upon cessation of treatment. Biochemical tests and urine analyses revealed no compound-related effects.

There were no macroscopic or microscopic findings post mortem that could be attributed to treatment with cyromazine. Some variations of organ weights were considered to be of equivocal biological significance in the absence of any cyromazine-related morphological alterations.

The NOAEL was 1000 ppm, equal to 36 mg/kg bw per day, for males on the basis of decreases in body-weight gains, food consumption and some haematological parameters in dogs fed 3000 ppm. In females, the NOAEL was 300 ppm, equal to 12 mg/kg bw per day, on the basis of a decrease in body-weight gain at 1000 ppm (Jessup, 1979).

In a study that complied with the principles of GLP (with QA certificate provided), groups of four male and four female beagle dogs were fed diets containing cyromazine (purity, 97.5%) at a concentration of 0, 50, 200, 800 or 3500 ppm for at least 52 weeks. The doses were equal to a mean daily intake of 0, 1.4, 5.7, 23 and 94 mg/kg bw per day in males and 0, 1.5, 6.0, 25 and 110 mg/kg bw per day in females. Mortality, clinical signs of toxicity, body weights and food consumption were monitored throughout the study. Ophthalmological examinations were conducted in all animals before dosing and at the end of treatment. Laboratory investigations (haematology, clinical chemistry and urine analysis) were performed on all dogs at intervals during the study (before the start of the test and after 4, 8, 13, 26 and 52 weeks). At the end of the scheduled period, the animals were killed and subjected to a postmortem examination. Terminal samples of blood and bone marrow were taken, selected organs were weighed and specific tissues were taken for subsequent histopathological examination. The study complied with OECD TG 452 (1981).

One female in the group at 3500 ppm was found dead on day 21 (week 3) and one male in the group at 200 ppm had to be sacrificed prematurely on day 197 (week 29) due to behavioural changes (aggressiveness). In the female, the clinical signs before death included weakness of the hindlimbs and blood in vomitus. This animal exhibited histopathological changes in the liver, kidneys and intestine. Overt signs in the male consisted of multiple injuries on the hind legs and muzzle and skin lesions on the scrotum. This dog also showed pre-terminal reduced food intake and subsequently loss in body weight. The reason for this behavioural change was unknown but was considered not to be treatment-related owing to its single appearance, its absence at higher doses and in the absence of histopathological changes in this dog. There were no other mortalities. In-life clinical signs consisted of sporadic appearance of vomitus in the group at the highest dose, the effect being more pronounced in females. Diarrhoea of a variable degree was observed in control and treated animals, but no dose–response relationship was evident. There were also isolated findings of injuries, hair loss, skin changes and blood in faeces, but in the absence of a dose–response relationship, these findings were considered to be incidental. Mean body weight was slightly decreased in females at 3500 ppm during the first weeks of treatment, but reached values similar to those for controls after about 12 weeks. Mean food consumption was slightly depressed in the females of this group during the first 5 weeks of treatment only, with the lowest food intake during week 1. After this period, food consumption was comparable to that of controls. The ophthalmological examinations did not reveal any treatment-related changes.

Haematology changes were confined to a slight, hypochromic and microcytic anaemia, characterized by lower values for haemoglobin concentration, erythrocyte volume fraction, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), and a tendency to lower erythrocyte counts, seen in males and females at 3500 ppm. Similar findings but without changes to MCV and MCH values were also seen in the males at 800 ppm. In addition, males at 800 ppm and 3500 ppm exhibited platelet counts that were higher than those for controls; however, they did

not differ appreciably from pre-test values. Males and females at 800 and 3500 ppm and males at 200 ppm had higher values for concentrations of plasma proteins associated with higher values for globulin and lower albumin-to-globulin ratios. In addition, lower mean triglyceride values and lower creatine kinase activities were seen in males at 3500 ppm and females at 3500 ppm had higher plasma chloride concentrations at weeks 26 and 52. Before termination, one female of the group at the highest dose exhibited increased activities for alkaline phosphatase, alanine aminotransferase and gamma-glutamyl transpeptidase; however, the changes revealed no dose–response relationship and were not related to duration of treatment. Values for other blood chemistry parameters in treated animals were similar to those of controls and/or pre-test values and the differences were of insufficient magnitude to be toxicologically relevant. Urine analysis revealed no treatment-related changes.

Increased absolute and relative heart (19/19% in males and 25/33% in females) and liver (15/15% in males and 29/37% in females) weights were noted in males and females at 3500 ppm. Increased relative heart and liver weights were also recorded in females at 800 ppm (15% and 12%, respectively) (Table 24). Relative kidney weights in females at the highest dose were also increased (12.5% compared with controls). The changes in heart and kidney weights in the group at the highest dose correlated with histopathological findings.

Table 24. Organ weights, macroscopic and microscopic findings in beagle dogs fed diets containing cyromazine for 1 year

Finding	Dietary concentration (ppm)									
	0		50		200		800		3500	
	M	F	M	F	M	F	M	F	M	F
Organ weights (% change)										
Heart,										
Absolute	—	—	—	—	—	—	—	—	+19%	+25%
Relative	—	—	—	—	—	—	—	+15%	+19%	+33%
Liver,										
Absolute	—	—	—	—	—	—	—	—	+15%	+29%
Relative								+12%	+15%	+37%
Kidney, relative	—	—	—	—	—	—	—	—	—	+12.5%
Macroscopic findings										
Heart, hard myocardium	—	—	—	—	—	—	—	—	3/4	2/4
Microscopic findings										
Heart										
Severe chronic myocarditis right atrium	—	—	—	—	—	—	—	—	3/4	2/4
Foci cartilaginous metaplasia in muscle	—	—	—	—	—	—	—	—	1/4	—

Kidney, focal chronic epithelial regeneration of renal tubules	—	—	—	—	—	—	—	—	2/4	2/4
Bone marrow, hypercellularity	—	—	—	—	—	—	—	—	4/4	2/4

From Altmann (1997)

F, female; M, male.

Macroscopic findings were limited to hard myocardium observed in three out of four males and in two out of four females at 3500 ppm (Table 24). Microscopic findings revealed severe chronic myocarditis in the right atrium of the heart in three out of four males and in two out of four females at 350 ppm. One male exhibited foci of cartilaginous metaplasia in the affected heart muscle. Focal chronic epithelial regeneration of the renal tubules was observed in the kidneys in two out of four males and two out of four females at 3500 ppm. Hypercellularity of the bone marrow was recognized in all males and in two out of four females at 3500 ppm.

Administration of cyromazine for 1 year to dogs produced effects at doses ≥ 200 ppm in males and ≥ 800 ppm in females. Target organs of toxicity were the heart, the kidney and the haematopoietic system on the basis of histopathological findings, organ-weight changes and/or haematology values. The NOAEL was 200 ppm, equal to 5.7 mg/kg bw per day, in males on the basis of haematological effects observed at the next higher dose and 800 ppm, equal to 25 mg/kg bw per day, in females on the basis of haematological effects and histopathological changes in heart and kidney observed at 3500 ppm. In the absence of biochemical or histopathological findings, the small increases in relative liver and heart weights observed in females at 800 ppm were not considered to be adverse (Altmann, 1997).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study that complied with the principles of GLP (with QA certificate provided), groups of 68 male and 68 female Charles River CD-1 mice were fed diets containing cyromazine (purity, 95.3–95.5%) at a concentration of 0, 50, 1000 or 3000 ppm for up to 2 years. The doses were equal to a mean daily intake of 0, 6.5, 126 and 384 mg/kg bw per day in males and 0, 8.2, 164 and 476 mg/kg bw per day in females. Eight male and eight female mice were assigned from each group for interim clinical pathology investigations after 52 weeks of treatment. The remaining mice were maintained on the study for 2 years. Samples of diet of all concentrations (including controls) were taken at intervals throughout the study and analysed for achieved concentration, stability and homogeneity. The mice were observed daily for appearance, behaviour, overt signs of toxicity, moribundity and mortality; detailed observations were recorded weekly. Individual body weights were obtained weekly during the initial 13 weeks of study and every 2 weeks thereafter. Individual food consumption was measured for 10 mice of each sex per group weekly for the first 13 weeks and every 2 weeks thereafter; test article consumptions were calculated using the body weight and food consumption measurements. Haematological tests were performed on eight randomly selected mice of each sex per group at 12 and 24 months. At the end of the scheduled period, the animals were killed and subjected to a full postmortem examination. Selected organs were weighed and specified tissues were taken for subsequent histopathology examination. The study was performed before OECD TG 451 (1981) had been enacted but complied to a great extent with these requirements.

Analysis of the diets showed that the stability of cyromazine in diets was satisfactory. Mean concentrations of cyromazine found in all diets analysed were 97 ± 31%, 102 ± 14% and 99 ± 9% of target concentrations at 50, 1000 and 3000 ppm, respectively. The homogeneity of cyromazine in the diet was improved in the second half of the study by a modification of the method by which the diet was prepared.

There was no clear dose-related effect on survival (62%, 63%, 55% and 55% in males and 52%, 47%, 57% and 40% in females from the control group, and at the lowest, intermediate and highest dose, respectively, at 104 weeks). Although the lowest rate of survival was in females at 3000 ppm, survival was within the expected range (values for historical controls for survival at 24 months were: males, 44.2%; females, 44.6%). There were no treatment-related differences noted in clinical condition between control and treated mice. Statistically significantly lower body weight and body-weight gains were noted for males at 3000 ppm throughout the study and for males at 1000 ppm from weeks 12 to 87. At week 104, the decreases in body-weight gain compared with controls were 12% and 18% at 1000 and 3000 ppm, respectively. Females at these doses showed occasional statistically significant differences in body weight and body-weight gain compared with controls; however, the differences were considered not to be biologically significant. At week 104, only slightly lower mean body weight was observed in treated animals compared with controls (0%, 2.4% and 7.1% in males and 2.9%, 5.7% and 5.7% in females). Food consumption was slightly reduced for males at 1000 and 3000 ppm throughout the study and was reduced in females at 3000 ppm, particularly in the first year of the study. There were no treatment-related effects on haematological parameters.

No treatment-related macroscopic changes were noted at the 12-month interim examinations. At study termination, there was a slightly increased incidence of masses in the livers of treated males compared with controls. There was no treatment-related effect on organ weights. A slight increase in relative liver weight in males at the highest dose was related to the reduced body weight in these animals.

Microscopic examination revealed a variety of neoplastic and non-neoplastic lesions. Except for hepatocellular neoplasms in male mice, the incidence and distribution of these lesions were similar to those of spontaneous lesions in CD-1 mice of a similar age. A slight increase in the frequency of hepatocellular neoplasms (adenomas and carcinomas) was noted in treated males (incidences of 18.3%, 28.8%, 32.1% and 26.3% in the control group and at the lowest, intermediate and highest dose, respectively) but, since there was no dose–response relationship, no increase in non-neoplastic proliferative lesions and no similar effect in females, it was not considered to be treatment-related (Table 25). Pulmonary metastasis was observed in one male mouse at 1000 ppm that had hepatocellular carcinoma. In females at the highest dose, the incidence of mammary gland adenocarcinomas was higher than in the other groups (4%, 8.3%, 5.7% and 16% in the control group and at the lowest, intermediate and highest dose, respectively). Data for historical controls indicated an expected range of 0–5.0% (mean, 1.3%). A variety of other neoplasms were observed in male and female mice in the control group and in mice receiving cyromazine. The incidence and distribution of these neoplasms were approximately equal in the control group and in groups receiving cyromazine. The most frequent of these were alveolar/bronchiolar adenomas and carcinomas of the lung, adenomas and carcinomas of the Harderian glands and malignant lymphoma involving primarily the thymus, spleen and lymph nodes.

Table 25. Incidence of hepatocellular neoplasms in CD-1 mice given diets containing cyromazine for 104 weeks

Finding	Dietary concentration (ppm)							
	0[a]		50		1000		3000	
	M	F	M	F	M	F	M	F
No. of animals examined	60	56	59	57	53	57	57	57
Liver								
Hepatocellular adenoma	5	0	9	2	11	1	5	3
Hepatocellular carcinoma	6	0	8	0	6	0	9	0
Hepatocellular adenoma and carcinoma	0	0	0	0	0	0	1	0
Mammary gland								
Adenocarcinoma[b]	—	2/2	—	7/4	—	3/3	—	9/8

From Blair (1982a)

F, female; M, male.

[a] Historical control data were obtained from 19 studies conducted at IRDC between 1976 and 1978 in 1490 males and 1490 females. Hepatocellular adenomas, mean: males, 2.3%; females, 0.9%; range, males, 0–26.7%; females, 0–6.0%. Hepatocellular carcinomas, mean: males, 8.4%; females, 1.0%; range, males, 1.0–26.0%; females, 0–10.0%. Mammary gland adenocarcinomas, females: mean, 1.3%; range, 0–5.0%.

[b] No. of lesions/No. of animals with lesions.

Generalized amyloidosis was observed in male and female mice in all test groups. The tissues most frequently involved were the small intestine, kidney, liver, adrenals, thyroid, heart and ovary. In the small intestine, amyloid was generally deposited in the lamina propria. In the adrenal gland, amyloid deposition was located at the corticomedullary junction. In the kidney and liver, amyloid deposition occurred in the renal glomerulus and around hepatic veins. Amyloid deposits in the thyroid occurred in the perifollicular tissue, while the deposits were scattered in the heart and ovary. Several male and female mice had chronic inflammatory lesions in the lungs. The severity of the lesion varied but was generally mild. These lungs had patchy areas with thickened alveolar walls lined by low cuboidal epithelium. This adenomatous change was frequently accompanied by intra-alveolar accumulation of alveolar macrophages and peribronchiolar and perivascular mononuclear cell infiltrates. The lesions resembled those associated with resolving pulmonary infections with Sendi virus in mice. Inflammatory lesions in the kidney were observed in mice of both sexes. Interstitial mononuclear infiltrates were observed in the renal cortex and a few mice had proteinaceous tubular casts, regenerative tubular epithelium and tubular cysts. Cystic endometrial hyperplasia was frequently seen in female mice in the control group and in groups receiving cyromazine and was characterized by proliferation of the epithelial lining of the uterus with cystic dilatation of endometrial glands. Parovarian cysts and cystic follicles were commonly observed in the ovaries of female mice in all groups. Inflammatory, degenerative and non-neoplastic proliferative lesions were less frequently observed in other tissues. Extramedullary hematopoiesis and brown granular pigment resembling haemosiderin were present in the spleen of most of the mice. These are common findings in the spleen of mice. Nematode parasites were present in the large intestines of a few animals. The haematopoietic activity of the bone marrow, as judged by overall cellularity, was within the normal range and no differences were noted among treatment groups. Other lesions were few in number and scattered among tissues in male and female mice in the control group and in groups receiving cyromazine.

In conclusion, dietary administration of cyromazine to CD-1 mice for up to 24 months resulted in a toxicologically relevant decrease in body-weight gain in males at 3000 ppm. The lower body weights observed in males at 1000 ppm and in females at 1000 and 3000 ppm were not

considered to be biologically significant owing to the very small differences between the means. Cyromazine did not produce inflammatory, degenerative or neoplastic lesions. A slightly higher number of hepatocellular neoplasms were observed in males receiving cyromazine, but were not considered to be treatment-related owing to the absence of non-neoplastic proliferative lesions, the lack of a dose-related response and the absence of similar changes in females. In females at the highest dose, the incidence of mammary gland adenocarcinomas was higher than in the other groups, and was above the upper limit of the range for historical controls. The NOAEL was 1000 ppm in males, equal to 126 mg/kg bw per day, on the basis of changes in body weight (Blair, 1982a).

Rats

In a study that complied with the principles of GLP (with QA certificate provided), groups of 60 male and 60 female Charles River CD rats were fed diets containing cyromazine (purity, 95.3–95.5%) at a concentration of 0, 30, 300 or 3000 ppm for up to 2 years. An additional 10 males and 10 females were initiated concurrently in the control group and in the group at 3000 ppm to allow for interim sacrifices; five rats of each sex were killed after 1 year and another five rats of each sex were fed control diet for a 4-week recovery period before termination after 1 year of dosing. The doses were equal to a mean daily intake of 0, 1.5, 15 and 156 mg/kg bw per day in males and 0, 1.8, 19 and 210 mg/kg bw per day in females. Samples of diet at all dietary concentrations (including controls) were taken at intervals throughout the study and analysed for achieved concentration, stability and homogeneity. The mice were observed daily for appearance, behaviour, overt signs of toxicity, moribundity and mortality; detailed observations were recorded weekly. Individual body weights were obtained weekly during the initial 13 weeks of the study and every other week thereafter. Individual food consumption was measured for 10 randomly selected rats of each sex per group weekly for the first 13 weeks and every other week thereafter; test article consumptions were calculated using the body weight and food consumption measurements. Haematological and clinical biochemistry (blood and urine) tests were performed on eight randomly selected rats of each sex per group before study initiation and at 6, 12, 18 and 24 months. At the end of the scheduled period the animals were killed and subjected to a full postmortem examination. Terminal samples of blood and bone marrow were taken, selected organs were weighed and specified tissues were taken for subsequent histopathology examination. The study was performed before OECD TG 453 (1981) had been enacted but complied to a great extent with these requiremets. The following deviations were noted: albumin was not measured, adrenals and ovaries were not weighed and ophthalmoscopy was not performed.

Analysis of the diets showed that the stability of cyromazine in diet was satisfactory. Diet analysis at the highest dose was satisfactory. At the lowest dose, higher variation in the content of cyromazine was observed in the period up to week 59. From week 60, the procedure was changed and concentrations were satisfactory for all doses. The mean concentrations of cyromazine found in all analysed diets were 96%, 101% and 99% of nominal concentrations at 30, 300 and 3000 ppm, respectively, indicating that animals had been adequately exposed.

There were no treatment-related differences noted in clinical condition, behaviour or survival between rats in the control group and rats treated with cyromazine. At termination of the study, survival was 60–67% in males and 53–70% in females. There was a decrease in mean body weight in both sexes at 3000 ppm (20.3% in males and 28.3% in females at week 104) compared with controls. Statistically significantly lower body-weight gain (22% and 33% in males and females, respectively at week 103) and food consumption (13.2% and 9.3% in males and females, respectively) were noted in both sexes at 3000 ppm. A statistically significant decrease in body-weight gain of 6–11% was also observed during weeks 12–55 in females at 300 ppm. Occasionally lower food consumption was observed in both sexes at 300 ppm. During the last 10 weeks of the study, the body weights of

females at the intermediate dose were comparable to those of the control group. The recovery animals of both sexes gained weight over the 4-week recovery period. Weight gains of 4–6% were noted for the control group, while rats at the highest dose in the recovery group exhibited weight gains of 11–19%.

There were no treatment-related differences between rats in the control group and rats treated with cyromazine noted in the clinical pathology parameters. There were no treatment-related differences noted in macroscopic pathology between rats in the control group and rats treated with cyromazine. Reductions in organ weights (liver, kidney, heart and brain) in rats at the highest dose were associated with reduced body weights in this group.

Tumours of the pituitary gland (adenoma and adenocarcinoma) in males and females, and mammary gland tumours in females, were seen in all groups including the controls (Table 26). There was a slightly higher incidence of testis interstitial cell tumours in males at the highest dose (6 out of 57 compared with 1 out of 60, 2 out of 59 and 1 out of 58 in the control group and at the lowest and intermediate dose, respectively) and mammary gland adenocarcinomas in females at the highest dose (9 out of 59 compared with 3 out of 53, 2 out of 58, and 1 out of 58 in the control group and at the lowest and intermediate dose, respectively). Both these values for incidence, as well as that for the incidence of pituitary gland adenomas and carcinomas, were within the range for historical controls in the laboratory. Other tumours occurred much less frequently and their incidence was not treatment-related.

Table 26. Incidence of selected tumours in rats fed diets containing cyromazine for 2 years

Tumour	Dietary concentration (ppm)							
	Males				Females			
	0[a]	30	300	3000	0[a]	30	300	3000
Pituitary								
No. of animals examined	60	56	57	57	55	59	56	58
Adenoma	26	19	19	17	29	31	38	42
Adenocarcinoma	7	12	8	5	15	11	7	11
Mammary gland								
No. of animals examined	56	46	43	55	53	58	58	59
Adenoma	0	0	0	1	3	8	6	8
Adenocarcinoma	0	0	2	0	3	2	1	9
Testis	60	59	58	57	—	—	—	—
Interstitial cell tumours	1	2	1	6	—	—	—	—

From Blair (1982b)

[a] Data on historical controls (16 2-year studies conducted at IRDC between 1975 and 1979, data from 1010 males and 1071 females). Pituitary adenoma: mean, 28.5%, range, 8.3–68.0% in males; and mean, 52.5%, range, 18.3–75.0% in females. Pituitary carcinoma: mean, 2.2%, range, 0–10.0% in males; and mean, 5.0%, range, 0–24.0% in females. Mammary gland adenomas: mean, 0.3%, range: 0–3.3% in males; and mean, 4.9%, range, 0–21.7% in females. Mammary gland carcinomas: mean, 0.3%, range, 0–1.7% in males; and mean, 9.5%, range, 1.5–21.4% in females. Interstitial cell tumours of testis: mean, 7.7%, range, 0–22.0%.

Non-neoplastic findings, such as inflammatory, degenerative, developmental and hyperplastic changes were mainly seen in the kidney, lung and liver (Table 27). In the kidney, there was evidence of chronic progressive nephropathy, commonly seen in older rats. Females fed diets containing cyromazine at 3000 ppm had a slightly higher incidence of pelvic epithelial hyperplasia (a common accompaniment of chronic nephropathy) compared with other groups. However, the incidence of chronic nephropathy was lower in the group at the highest dose than in other groups. Therefore, the

observed incidence probably related to variability in degree of change as a result of ageing and was not treatment-related. Chronic respiratory disease, characterized by bronchiectasis, suppurative bronchitis and cellular infiltrates or proliferations, was evident in the lung, with a slightly higher incidence in males at 3000 ppm compared with controls. However, this finding is common and the incidence was variable, therefore it may not have been a result of treatment with cyromazine. Microscopic changes in the liver were of a type and incidence common in older rats of this strain. All the neoplastic and non-neoplastic changes observed were considered to be spontaneous lesions, with no direct evidence of a treatment-related incidence and within the normal range of variations.

Table 27. Incidence of selected microscopic findings in rats fed diets containing cyromazine for 2 years

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	30	300	3000	0	30	300	3000
Kidney								
Chronic nephropathy	41	43	40	22	14	14	11	2
Epithelial hyperplasia	1	3	0	2	1	9	6	15
Lung								
Hyperplasia	21	13	18	19	11	8	9	11
Suppurative bronchitis	16	11	13	23	9	12	6	10
Bronchiectasis	7	4	6	18	2	5	4	10
Liver								
Pericholangitis	8	11	3	6	7	12	9	3
Vacuolation	0	0	0	0	1	1	0	0
Focal vacuolation:	6	2	2	2	1	1	0	0
Hepatocytomegaly	0	0	0	0	2	5	5	3
Focal hepatocytomegaly	1	2	2	2	1	5	3	5

From Blair (1982b)

In conclusion, dietary administration of cyromazine for up to 2 years resulted in decreased body-weight gain, lower mean body weight and food consumption in male and female rats at 3000 ppm. On the basis of these effects, the NOAEL was 300 ppm, equal to 15 mg/kg bw per day in males and 19 mg/kg bw per day in females. In females, a higher incidence (but within the range for historical controls) of mammary gland tumours was observed at 3000 ppm (Blair, 1982b).

2.4 Genotoxicity

The mutagenic/genotoxic potential of technical cyromazine was investigated in a battery of tests in vitro and in vivo (Table 28). All the results were negative, with the exception of an inconclusive spot test in mice. The Meeting considered that cyromazine is not genotoxic.

Table 28. Results of studies of genotoxicity with cyromazine

End-point	Test object	Concentration[o]	Purity (%)	Results	GLP or QA	Reference
In vitro						
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	20–5000 µg/plate, in DMSO	97.5	Negative[a,b]	GLP & QA	Deparade (1988)
Reverse mutation	*S. typhimurium* TA1538 & E. coli WP2*uvrA*	20–5000 µg/plate, in DMSO	97.5	Negative[a,c]	GLP & QA	Deparade (1990)
Gene mutation, mitotic gene conversion & mitotic recombination	*Saccharomyces cerevisiae D7*	375–3000 µg/ml, in DMSO	98.9	Negative[a,d]	GLP & QA	Hool (1984)
Gene mutation	Mouse lymphoma cells, L5178Y, $Tk^{+/-}$	50–500 µg/ml, in culture medium, 4-h exposure	96.2	Negative[a,e]	GLP & QA	Beilstein (1985)
Gene mutation	Chinese hamster, V79 cells, *Hprt* locus	25–1000 µg/ml, −S9, in ethanol, 21-h exposure 100–4000 µg/ml, +S9, in ethanol, 5-h exposure	98.9	Negative[a,f]	GLP & QA	Dollenmeier (1986)
Chromosomal aberration	Human peripheral blood lymphocytes	62.5, 125, 250, 500 and 1000 µg/ml in DMSO, ± S9, 3-h treatment, harvesting 46 h after the end of treatment	96.2	Negative[a,g]	GLP & QA	Strasser (1985)
Unscheduled DNA synthesis	Rat (F344) primary hepatocytes	$1–10^{-4}$ mg/ml in DMSO, 18-h exposure	NR	Negative[h]	QA	Tong (1982)
Unscheduled DNA synthesis	Mouse (male CD-1) primary hepatocytes	$1–10^{-4}$ mg/ml in DMSO, 18-h exposure	NR	Negative[i]	QA	Tong (1983)
In vivo						
Nucleus anomalies[j] (BM cells)	Chinese hamster (three male + three female/group)	Two oral doses at 2000, 4000 and 8000 mg/kg bw (24 h apart) in CMC	98.9	Negative[k]	—	Hool (1980)
Micronucleus test (BM cells)	Mouse (Tif:MAGf SPF, NMRI derived) (five male + five female/group)	Single oral doses of 360 and 1080 mg/kg bw in CMC-Sampling time: 24, 48 and 72 h after treatment	96.3	Negative[l]	GLP & QA	Strasser (1987)
Spot test	Mouse (males: T-stock, females: C57Bl/6)	Single intraperitoneal. doses at 150, 300 and 600 mg/kg bw in sesame oil	96.2	Inconclusive[m]	GLP & QA	Strasser (1986)
Dominant lethal	Mouse (Tif:MAGf SPF, NMRI derived)	Single oral doses at 226 or 678 mg/kg bw in PEG 400	98.9	Negative[n]	—	Hool (1981)

CMC, carboxymethyl cellulose; DMSO, dimethyl sulfoxide; GLP, good laboratory practice; NR, not reported; QA, quality assurance; S9, S9, 9000 × *g* supernatant from livers of male rats.

[a] With and without metabolic activation.

[b] Cyromazine was 264assayed twice using the standard plate incorporation protocol over a dose range of 20-5000 µg/plate, ±S9 prepared from Aroclor-induced male RAI rats. The experimental protocol essentially complied with OECD TG 471 (1997).

[c] Cyromazine was assayed twice using the standard plate incorporation protocol over a dose range of 20-5000 µg/plate, ±S9 prepared from Aroclor-induced male RAI rats. The experimental protocol essentially complied with OECD TG 471 (1997).

[d] Cyromazine was tested ±S9. Two independent repeat tests were performed. Positive controls: –S9, 4-nitroquinoline-*N*-oxide; +S9, cyclophosphamide. The highest concentration of test substance was chosen on the basis of the solubility of the test substance and caused no decrease in survival of the yeast cells. Experimental protocol essentially in complied with OECD TG 480 and TG 481 (1986).

[e] Cyromazine was tested ±S9. Two independent repeat tests were performed. Positive controls: –S9, ethylmethane sulfonate; +S9, dimethylnitrosamine. Cyromazine produced no cytotoxicity at the doses tested. The experimental protocol was essentially in compliance with OECD TG 476 (1997).

[f] Cyromazine was tested ±S9. Two independent repeat tests were performed. Positive controls: −S9 mix, ethylmethane sulfonate; +S9, dimethylnitrosamine. In a preliminary assay, toxicity was observed −S9 but not +S9. Therefore, 4000 µg/ml was chosen as the highest concentration for the main assay + S9 and 2000 µg/ml −S9. The experimental protocol was essentially in compliance with OECD TG 476 (1997).

[g] Cyromazine was tested ±S9. Positive controls: −S9, mitomycin C; +S9, cyclophosphamide. Concentration-related reductions in mitotic activity were observed in cultures in a preliminary assay for cytotoxicity. The highest concentration chosen for the main assay was 1000 µg/ml (mitotic index, about 68% of control values in preliminary tests for cytotoxicity test). The experimental protocol was not totally in compliance with OECD TG 473 (1997) as a repeat test was not performed and treatment −S9 should have been for a cell cycle.

[h] Positive control was 7,12-dimethylbenzanthracene and negative control was anthracene. Unscheduled DNA synthesis (UDS) was measured by autoradiography. The highest dose tested was limited by the solubility of the test material. Cytotoxicity was observed in one of the cultures at the highest concentration (1 mg/ml) tested. The test protocol was not in compliance with OECD TG 482 (1986) as only a single test was performed and less than 50 cells per culture were evaluated for UDS. The study was performed before the GLP certification of laboratories, but was conducted according to the principles and practices of GLP.

[i] Positive control was benzo(a)pyrene and negative control was pyrene. UDS was measured by autoradiography. Three independent repeat assays were performed. No cytotoxicity was observed in any of the tests at the highest concentration (1 mg/ml) tested. The highest concentration was determined by the solubility of the test substance. The test protocol was not in compliance with OECD TG 482 (1986), as only 40 cells per culture were evaluated for UDS instead of 50. The study was performed before the GLP certification of laboratories, but was conducted according to the principles and practices of GLP.

[j] Single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leukopoietic cells and polyploid cells.

[k] Cyclophosphamide was used as positive control. Two females died after the first application of vehicle control. In the group receiving the highest dose, two females died after the second dose. The study was performed before OECD TG 474 was enacted. Less than 1000 polychromatic erythrocytes (PCE) were analysed for the presence of micronuclei. Exact number of erythrocytes was not stated in the report. Only three animals per sex were analysed.

[l] Cyclophosphamide was used as positive control. The highest dose was selected on the basis of a tolerability test showing that no deaths occurred in a group of four animals. In the test for mutagenicity, one male at the highest dose died within 72 h. The experimental protocol was not totally in compliance with OECD TG 474 (1997) as only two doses were used. Only 1000 polychromatic erythrocytes (PCE) were analysed for the presence of micronuclei.

[m] Each group consisted of 48 males and 96 females. Each untreated male was placed in a cage with two untreated females. After successful mating, females were removed from the mating cages before allocation to treatment groups. Groups of 71 presumed pregnant animals received a single intraperitoneal injection of cyromazine at the appropriate dose on day 10 after conception. Examination of the offspring for spots was started at age 12–14 days and was performed twice per week between weeks 3 and 5 after birth. The incidence of recessive spots (RS) and white mid-ventral spots (WMVS) was recorded. Total numbers of offspring examined for each group were 354, 354, 357 and 93 for the control group and the group at the lowest intermediate and highest dose, respectively. In a preliminary test, 600 mg/kg bw was found to be the highest dose not causing deaths in groups of non-pregnant female mice. At 600 mg/kg bw, a decrease in the average litter size was observed (4.33 compared with 6.61, 7.52 and 6.88 in the vehicle control group and at the lowest and intermediate dose, respectively). Survival up to the start of spot observation was reduced at the two highest doses. There was a statistically significant increase in the incidence of RS at doses of 300 (1.12%) and 600 mg/kg bw (2.15%) when compared with the concurrent control group (0%). However, owing to the low incidences of RS and the marked reduction in litter size in the group at the highest dose, it was necessary to compare the groups at the two higher doses with the cumulative negative controls using the Fischer exact test. In this test, a dose at 300 mg/kg bw

or 600 mg/kg bw did not differ significantly from the data for historical controls (RS, 0.75%) derived from experiments with the appropriate vehicle (sesame oil) alone ($p = 0.514$ and $p = 0.179$ for the groups at 300 and 600 mg/kg bw). By contrast, cumulative positive control experiments with ethylmethanesulfonate (100 mg/kg bw) yielded an average RS frequency of 1.41%. This was significantly different (Fisher exact test for RS, $p = 0.013$) from the data for historical controls (RS, 0.26%) derived from experiments with the appropriate vehicle (physiological saline or Hank BSS) alone. It was therefore concluded by the performing laboratory that the observed increase was not indicative of a mutagenic effect. The Meeting considered the study inconclusive as interpretation of the results was confounded by reduced reproductive performance of the group at the highest dose, resulting in a much smaller number of observations. The study protocol complied with OECD TG 484 (1986).

[n] Groups of 20 fertile males were treated with cyromazine. Approximately 8 h after dosing, each of the males was placed in a cage with two untreated females. After 1 week, the females were removed and replaced by another two females. This procedure was continued for 6 consecutive weeks in order to examine the effect of treatment on all stages of the spermatogenic cycle. The females were examined daily for the presence of a vaginal plug which would indicate successful mating. The day that a vaginal plug was observed was designated as day 0 of gestation. The uteri of the females were examined for live implantations, early deaths and late deaths 14 days after they were first housed with the males. Three males died within 24 h after treatment with the compound at the highest dose. The data on mating ratio, on the number of implantations and embryonic deaths are comparable for all groups. A study with a positive control, thiotepa, was carried out in 1976. This was administered in a single intraperitoneal dose at at doses of 3.65 or 11 mg/kg to male mice. These males were then housed with untreated females for 1 week. At the end of the week the females were replaced by new ones. This procedure was continued for 8 consecutive weeks. A cytotoxic and dominant lethal effect was observed in the progeny of male mice treated with thiotepa. The study complied to a great deal with OECD TG 478 (1984), except that there were only two treated groups plus controls and a positive-control study, carried out within 3 years of the main study, was reported in a supplement to the main report. This study was performed before GLP guidelines were enacted and therefore not performed under formal quality assurance, but a compliance statement was provided. Preimplantation losses could not be accurately calculated as the number of corpora lutea was not recorded.

[o] Vehicle was used as negative control. Positive control substances were used in all assays and gave the expected results.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a study that complied with the principles of GLP (with QA certificate provided), groups of 15 male and 30 female (F_0 parents) weanling Sprague-Dawley COBS CD rats were fed diet containing cyromazine (purity, 95.3%) at a concentration of 0, 30, 1000 or 3000 ppm (initially 4000 ppm, reduced to 3000 ppm after 4 weeks of feeding to the F_0 parents). The doses were equal to a mean daily intake of 0, 2.0, 64 and 228 mg/kg bw per day in F_0 males and 0, 2.3, 77 and 259 mg/kg bw per day in F_0 females, and 0, 1.6, 51 and 169 mg/kg bw per day in F_1 males and 0, 1.9, 66 and 202 mg/kg bw per day in F_1 females. After 100 days, the animals were mated and allowed to rear the ensuing F_1 litters to weaning. From these weanlings, the F_1 parents were selected and after a 120 day pre-mating period were mated to produce the F_2 litters. In each generation, on day 4 of lactation, the litters were reduced to 10 animals (where possible, of equal sex ratio). Test diets were fed continuously throughout the study. Body weights, food consumption (measured only during pre-mating periods), fertility and the general appearance, behaviour and survival of all parental rats were observed and recorded during the study. Female parental rats were also observed for duration of gestation and any difficulties during parturition. The litter parameters evaluated and recorded included pup viability on day 0 of lactation, mean litter size, pup body weights and survival during lactation and the general appearance and behaviour of the pups. Complete gross and histopathological postmortem examinations were conducted on all parental rats (F_0 and F_1 generations), on five F_1 weanlings of each sex per group not selected as members of the F_1 generation and on five F_2 weanlings of each sex per group. The remaining F_1 and F_2 weanlings were given gross internal examinations only if appearing abnormal on external examination. Those appearing normal were discarded. Samples of diet at all concetrations (including controls) were taken at intervals throughout the study and analysed for achieved concentration. Stability and homogeneity were measured.

The study was performed before OECD TG 416 (1983) was enacted and did not comply entirely with these requirements. Among points on which it failed to comply were: females were housed with males for up to three 10-day periods, food consumption was measured during the pre-mating periods only, F_1 offspring not selected for mating were as old as 30 days at postmortem examination, cervix, vagina and seminal vesicles of all animals selected for histopathology were not listed in the report as being examined, individual weights of the pups at termination have not been reported. The Meeting considered that these deviations did not compromise the scientific validity of the study.

In the early part of the study, analytical results suggestive of non-homogeneity of the diet containing cyromazine at 30 ppm were noted; results ranged from 11% of the target (average of the duplicate assays at study week 23) to 133% (study week 15). Prepared diets containing cyromazine at 1000 and 3000 ppm contained 92–143% and 72–125% of the desired concentrations, respectively. The mean concentrations found from study weeks 1–25 were 78 ± 29%, 109 ± 12% and 104 ± 9.1% of the target levels in the groups receiving cyromazine at 30, 1000 and 3000 ppm, respectively. In diets prepared for study weeks 26 to termination, concentrations of cyromazine ranged from (mean ± SD) 47% to 155% (91 ± 23%), 83% to 122% (101 ± 9.6%) and 76% to 116% (97 ± 9.6%) of the target levels in the groups at 30, 1000 and 3000 ppm; respectively. Nevertheless, although there were notable variations in concentrations in diet at 30 ppm in the first part of the study, the overall exposure was considered to be acceptable. The overall range and mean of doses received during the pre-mating period is reported in Table 29.

Table 29. Overall range and mean of dose received during the pre-mating period in a two-generation reproduction study in rats fed diets containing cyromazine

Generation/sex	Dietary concentration (ppm)					
	30		1000		3000	
	Dose received (mg/kg bw per day)					
	Range	Mean	Range	Mean	Range	Mean
F_0						
Males	3.09–1.39	1.97	101–43.7	64.1	368–157	228
Females	3.28–1.87	2.34	108–61.9	77.2	398–192	259
F_1						
Males	2.00–1.28	1.55	66.8–43.0	51.3	278–137	169
Females	2.37–1.66	1.94	81.2–57.2	66.3	246–169	202

From Blair (1981a)

One female F_1 parental rat died but the death was considered to be incidental to treatment with cyromazine. Survival was 100% in the remaining animals in both generations. The clinical condition of parental animals was not affected by treatment with cyromazine; however, there was some evidence of a viral infection affecting a small number of rats (sialodacryoadenitis virus, SDV). Dose-related and statistically significant decreases in parental mean body weight were observed in males at 1000 and 3000 ppm and females in the F_0 generation and in F_1 females as well as in F_1 males at 3000 ppm. In the F_0 generation, mean body weights were reduced by 7.5% and 17.5% respectively, for males, and 8.6% and 17.0% respectively, for females, compared with controls. In the F_1 males at 3000 ppm, mean body weights were reduced by approximately 14.5% compared with controls. Food consumption nearly paralleled the body-weight effect with slight decreases in the group at 1000 ppm group and moderate decreases in males and females of both generations at 3000 ppm.

The reproductive performance of rats treated with cyromazine at all doses was comparable with that of controls for both matings. Male fertility in the group at 3000 ppm of the F_0 generation was reduced and may have been compound-related (Table 30). Male and female fertility in the group at 30 ppm of the F_1 generation was reduced when compared with the control groups, but these observations were not consistent across generations and there was no evidence of a dose–response relationship; therefore it was not considered to be treatment-related. There were no remarkable differences in the process of parturition or the duration of gestation between females receiving cyromazine and those in the control group for either generation.

Table 30. Fertility index in a two-generation study of reproduction in rats fed diets containing cyromazine

Generation	Fertility index (%)							
	Dietary concentration (ppm)							
	Males				Females			
	0	30	1000	3000	0	30	1000	3000
F_0	86.7	86.7	92.9	66.7	80.0	90.0	90.0	76.7
F_1	86.7	66.7	100.0	93.3	86.7	76.7	86.7	90.0

From Blair (1981a)

There was no evidence of any effect of treatment on macroscopic changes on F_0 parents, F_1 adults or weanlings or F_2 pups. Occasional statistically significant differences between control and treated groups were noted in relative organ weights. However, these changes were considered to be

either sporadic or as a consequence of reduced body weights at 3000 ppm, and of no toxicological significance. There were no treatment-related microscopic findings.

Pup viability at birth and mean litter size in the treated groups of the F_1 litters were similar to control. In the F_2 litters, pup viability at birth and mean litter size were reduced in the group at 3000 ppm (decrease of 5% and 7.9%, respectively); subsequent survival was not affected. Mean pup body weight in both generations was reduced at 3000 ppm.

Dietary administration of cyromazine to rats for two generations resulted in decreased parental body weights and food consumption at doses of 1000 and 3000 ppm and decreased pup body weights at 3000 ppm. The effects at 1000 ppm were not considered to be biologically significant. Male fertility was reduced in the F_0 generation at 3000 ppm; however, reproductive performance was not affected. A decrease in pup viability at birth and mean litter size was also observed in F_2 litters at 3000 ppm. The NOAEL for parental toxicity, reproduction and offspring toxicity was 1000 ppm, equal to 64 mg/kg bw per day in males and 51 mg/kg bw per day in females (Blair, 1981a).

(b) Developmental toxicity

Rats

In a study that complied with the principles of GLP (with QA certificate provided), groups of 20 pregnant Charles River COBS®CD® rats were given cyromazine (purity, 96.3%) at a dose of 0, 100, 300 or 600 mg/kg bw per day, by gavage in 1% (w/v) aqueous CMC, on days 6–19 (inclusive) of gestation. The day that evidence of mating was detected was designated as day 0 of gestation. Before treatment, the dams were observed daily for mortality and overt changes in appearance and behaviour. They were observed daily for mortality and clinical signs of toxicity on days 6–20 of gestation. Individual maternal body weights were recorded on days 0, 6, 9, 12, 16 and 20 of gestation. On day 20 of gestation, the rats were killed and their uteri weighed and examined for live fetuses and intrauterine deaths. The abdominal cavities and organs of the dams were examined for grossly evident morphological changes. The fetuses were weighed, examined for external abnormalities and sexed. Approximately one third of the fetuses were examined for visceral abnormalities by razor-blade sectioning and the remaining two thirds were stained for skeletal examination. The study was performed before OECD TG 414 (1981) had been enacted and did not comply entirely with these requirements, e.g. food consumption was not recorded and visceral examination was carried out on one third of all fetuses. The Meeting considered that these deviations did not compromise the scientific validity of the study.

Clinical signs seen in the dams at 600 mg/kg bw per day included increased activity during the first portion of the treatment period, red nasal discharge, clear oral discharge and inactivity beginning mid-way through the treatment period. Red nasal discharge was also seen in the group at 300 mg/kg bw per day. Maternal body-weight gain was moderately reduced in the group at 600 mg/kg bw per day (days 6–20 of gestation, 69.3%; and days 0–20 of gestation, 73.5% of controls, respectively) and slightly reduced in the group at 300 mg/kg bw per day (days 6–20 of gestation, 82.5%; and days 0–20 of gestation, 85.7% of controls, respectively). A mean maternal body-weight loss occurred in both groups during the first 3 days of treatment.

There were no biologically meaningful or statistically significant differences in the mean numbers of viable fetuses, early or late resorptions, total implantations or numbers of corpora lutea. There were no biologically meaningful or statistically significant differences in the fetal sex distribution or the number of litters with malformations in any of the treatment groups. A slight decrease in mean fetal body weight in the group at 300 mg/kg bw per day (3%) and a statistically significant decrease in the group at 600 mg/kg bw per day (6%) was noted. A definite increase in fetuses with reduced ossification was noted at 600 mg/kg bw per day (Table 31). Increases occurred

in the number of litters and fetuses with developmental variations in all treated groups. Slight increases were noted in reduced ossification of the skull in all groups and in unossified sternebrae (No. 5 and/or No. 6 and other sternebrae) in the groups at 100 and 300 mg/kg bw per day. These very slight increases in the group at 100 mg/kg bw per day may have been due to random occurrence as there were no other signs of fetal toxicity. A definite increase was noted in unossified sternebrae in the group at 600 mg/kg bw per day.

Table 31. Incidence of skeletal variations of fetuses in a study of developmental toxicity in rats given cyromazine by gavage

Finding	Dose (mg/kg bw per day)			
	0 (control)[a]	100	300	600
No. of. litters examined	23	22	25	24
No. of fetuses examined skeletally	224	217	238	224
Skull, reduced ossification (No. of litters affected)	0 (0)	2 (1)	1 (1)	2 (2)
Hyoid unossified (No. of litters affected)	5 (4)	3 (3)	3 (2)	0 (0)
Vertebrae, reduced ossification (No. of litters affected)	1 (1)	2 (2)	1 (1)	1 (1)
Pubis unossified (No. of litters affected)	0 (0)	0 (0)	1 (1)	0 (0)
Metetarsals unossified (No. of litters affected)	0 (0)	0 (0)	1 (1)	0 (0)
Sternebrae No. 5 and/or No. 6 unossified (No. of litters affected)	48 (17)	63 (19)	79 (22)	171 (23)
Other sternebrae unossified (No. of litters affected)	2 (2)	5 (4)	5 (4)	24 (11)

From Rodwell (1979)

[a] IRDC reported incidence of sternebrae No. 5 and/or No. 6 unossified among historical controls: 12.5% of the fetuses in 45.7% of the litters.

Cyromazine was not teratogenic in rats given doses of up to 600 mg/kg bw per day by gavage. Signs of maternal toxicity (clinical signs of toxicity and decreased body-weight gain) were observed at 300 and 600 mg/kg bw per day and fetal toxicity (decreased body weight and reduced ossification) was observed in the group at the highest dose. The NOAEL for maternal toxicity was 100 mg/kg bw per day and the NOAEL for developmental toxicity was 300 mg/kg bw per day (Rodwell, 1979).

Rabbits

Groups of 16 artificially inseminated Dutch Belted rabbits were given cyromazine (purity, 96.3%) as a single daily dose at 0, 25, 50 or 75 (experiment 1) and 0, 10, 30 or 60 mg/kg bw per day (experiment 2) a constant volume of 1 ml/kg (in 1% (w/v) in aqueous CMC) orally by gavage on days 6–27 (inclusive) of gestation. The day of artificial insemination was designated day 0 of gestation. Before treatment, the females were observed daily for mortality and overt changes in appearance and behaviour. The females were observed daily for mortality and clinical signs of toxicity on days 6–28 of gestation. Dams not surviving to the scheduled sacrifice were necropsied and fetuses from these dams were examined externally and preserved in 10% neutral buffered formalin. Any female showing signs of abortion was sacrificed and examined for grossly evident morphological changes. Intact fetuses were examined externally and preserved in 10% neutral buffered formalin. Individual maternal body weights were recorded on days 0, 6, 12, 18, 24 and 28 of gestation. On day 28 of gestation, all surviving females were killed and the uterus was excised and weighed. The number and location of viable and non-viable fetuses, early and late resorptions, number of total implantations and corpora lutea were recorded. The dams were examined macroscopically and maternal tissues were preserved for microscopic examination only as deemed necessary by the gross findings. All

fetuses were removed, weighed and examined for external malformations. Each fetus was dissected, internally sexed and examined for visceral abnormalities and then processed for subsequent skeletal examination. The study was conducted according to the principles and practices of GLP (with QA certificate) and was performed before OECD TG 414 (1981) had been enacted but complied to a great extent, except that food consumption was not measured.

Four, two, two and one animals died in the groups at 75, 60, 50 and 25 mg/kg bw per day, respectively. There were no mortalities in the groups at 10 or 30 mg/kg bw per day or in the controls. Nine females aborted (before death or where sacrificed) between day 20 and day 27 of gestation (two each in the groups at 30, 50 and 60 mg/kg bw per day and three in the group at 75 mg/kg bw per day). In experiment 2, a reduction in the amount of faeces was seen in the treated groups at various intervals during the treatment period. This finding occurred for a longer duration with increasing dose. Macroscopic examination of the dams post mortem revealed heart failure as a cause of death for two females, one each in the groups at 25 and 75 mg/kg bw per day; lung congestion and oedema were cited as possible causes of death for two females in the group at 60 mg/kg bw per day and pneumonitis-pleuritis was determined a cause of death for one dam at 75 mg/kg bw per day. There were no treatment-related trends in necropsy findings in either experiment when compared with those for the respective control groups.

In experiment 1, mean maternal body-weight losses were observed primarily during days 6 to 12 of gestation at 25 mg/kg bw per day (–101 g) and days 6 to 18 at 50 (–261 g) and 75 (–399 g) mg/kg bw per day when compared with the control groups. This resulted in mean maternal body-weight losses over the entire treatment period in these three groups. In experiment 2, there were moderate body-weight losses during days 6 to 18 of gestation in the groups at 30 (–133 g) and 60 (–255 g) mg/kg bw per day, resulting in a slight decrease in mean maternal body-weight gain at 30 mg/kg bw per day and a slight loss in mean maternal body weight at 60 mg/kg bw per day, for the entire study period. There was no effect on body-weight gain at 10 mg/kg bw per day.

In experiment 1, the pregnancy rates in all groups, including controls, were considerably reduced (12, 9, 10 and 10 females in the groups at 0, 25, 50 and 75 mg/kg bw per day, respectively) when compared with values for historical controls (82%) for this strain of rabbit (which may have resulted from a technician error when inseminating).

In experiment 1, there were no effects on the mean number of corpora lutea or mean fetal body weight in the groups at 25, 50 or 75 mg/kg bw per day or on mean postimplantation loss and fetal sex distribution in the groups at 25 or 50 mg/kg bw per day. A slight decrease in the mean number of total implantations (19.4%) and an increase in mean postimplantation loss (400%) with a corresponding and statistically significant decrease in the mean number of viable fetuses (50.8%) was observed at 75 mg/kg bw per day. Malformations were not observed in the 11 litters examined in the control group or in the eight litters examined in the group at 50 mg/kg bw per day. The only malformations observed were fused sternebrae in one fetus from one litter (of eight litters examined) in the group at 25 mg/kg bw per day and fetal anasarca in two fetuses from one litter (of four litters examined) in the group at 75 mg/kg bw per day.

In experiment 2, there were no biologically meaningful or statistically significant differences in the mean numbers of corpora lutea, total implantations, postimplantation loss, viable fetuses or the total sex distribution in any of the treated groups when compared with the control group. Although the mean fetal body weight was slightly reduced in all groups, this finding was considered to be unrelated to treatment since there was both a wide deviation within each group and a slight increase in the number of viable fetuses in the treated groups. Although there were no malformations observed in the control group in this experiment, the genetic and developmental variations seen in the treated groups were comparable to those cited for historical controls. In only one out of 11 recently documented studies were no malformations observed in the controls. Anomalies were observed in all the treated groups (5.3%, 6.7% and 12.1% of fetuses and 33.3%, 30.8% and 40.0% of litters affected at 10,

30 and 60 mg/kg bw per day, respectively). This finding was statistically significant ($p < 0.05$) in the group at the highest dose. However, most of these anomalies occurred only once and had been observed in historical controls. The number of fetuses with anomalies at incidences that exceeded values for historical controls included the following: two with carpal flexure at 10 mg/kg bw per day, one with fused sternebrae and one with malformed sternebrae at 30 mg/kg bw per day and one with internal hydrocephaly/dome-shaped head, one with skull anomalies (malformed nasals, premaxillae and jugals), one forked thoracic rib and three with fused sternebrae at 60 mg/kg bw per day (Table 32). A slight increase in the number of fetuses and litters with 27 presacral vertebrae and a 13th full rib was noted in the treated groups compared with the control group.

Table 32. Incidence of malformations and variations of fetuses (litters) in a study of developmental toxicity in rabbits given cyromazine by gavage—experiment 2

Finding	No. of fetuses affected (No. of litters affected)				
	Dose (mg/kg per day)				
	0 (control)	10	30	60	Historical controls
Total No. examined	77 (14)	95 (15)	90 (13)	58 (10)	951 (149)
External malformations					
Carpal flexure	0 (0)	2 (2)	1 (1)	0 (0)	4 (4)
Abdominal closure defects—omphalocele	0 (0)	0 (0)	0 (0)	2 (1)	2 (2)
Soft tissue malformations					
Internal hydrocephaly/ dome-shaped head	0 (0)	1 (1)	0 (0)	1 (1)	3 (3)
Skeletal malformations					
Scoliosis with/without rib anomaly	0 (0)	1 (1)	0 (0)	1 (1)	7 (7)
Fused sternebrae	0 (0)	1 (1)	1 (1)	3 (3)	1 (1)
Malformed sternebrae	0 (0)	0 (0)	1 (1)	0 (0)	1 (1)
Variations[a]					
Twenty-seven presacral vertebrae	5.2 (14.3)	8.4 (26.7)	10.0 (38.5)	20.7 (50.0)	68 (36)[b]
Thirteenth full rib	7.8 (21.4)	20.0 (46.7)	21.1 (69.2)	32.8 (70.0)	97 (44)[c]

From Blair (1981b)

[a] Percentage affected: fetuses (litters).

[b] Range, 1.8–14.1 (13.3–44.4).

[c] Range, 1.1–32.7 (7.7–70.0).

In conclusion, oral administration of cyromazine to pregnant Dutch Belted rabbits results in maternal toxicity at doses ≥ 25 mg/kg bw per day as shown by maternal deaths, abortions and decrease in body weight. In the first experiment, embryotoxicity was observed at 75 mg/kg bw per day (decreased total implantations, increased postimplantation losses and decrease in the number of viable fetuses), but no teratogenic effect was observed. In the second experiment, an increased incidence of malformations at above the range for historical controls were seen; however, their biological significance is uncertain. Factors relating their occurrence to treatment included the fact that a majority involved the sternum, they occurred in all treatment groups and their frequency of occurrence at 30 and 60 mg/kg bw per day exceeded values for historical controls. However, they occurred generally as single incidences.

The NOAEL for maternal toxicity was less than 25 mg/kg bw per day in the first experiment (the lowest dose tested) and 10 mg/kg bw per day in the second experiment. The NOAEL for developmental toxicity was 50 mg/kg bw per day in the first experiment on the basis of the decrease in viable fetuses and increase in postimplantation loss at 75 mg/kg bw per day, and 30 mg/kg bw per day in the second experiment on the basis of an increase in postimplantation loss and increased incidences of 27 presacral vertebrae at 60 mg/kg bw per day (Blair, 1981b).

In a second study, conducted in the same strain of rabbit and at the same laboratory, an enteric disease precluded a reliable evaluation of the effects observed. Groups of 18 inseminated rabbits were given cyromazine (purity not reported) at a dose of 0 (vehicle control, 1% CMC), 5, 10, 30 or 60 mg/kg bw per day by gavage on days 7–19 (inclusive) of gestation.

Cyromazine did not elicit developmental toxicity in this study at up to the highest dose tested, 60 mg/kg bw per day. The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of body-weight loss at ≥ 30 mg/kg bw per day during the treatment period. A slight reduction in food consumption was observed at 60 mg/kg bw per day only (Schardein, 1985).

In a third study, groups of 18 artificially inseminated New Zealand White rabbits (BUK(CRL) NZW FBR) were given cyromazine (purity, 95.2%) at a dose of 0 (environmental control, undosed), 0 (vehicle control, 0.5% aqueous CMC), 5, 10, 30 or 60 mg/kg bw per day by gavage on days 7–19 (inclusive). Throughout gestation, all females were observed twice per day for signs of toxicity. Individual maternal body weights were recorded on days 0, 7, 10, 14, 20, 24 and 29 of gestation and food consumption was recorded daily from days 0–29 of gestation. On day 29 of gestation, all surviving females were sacrificed and uteri examined for live fetuses and intrauterine deaths. All fetuses were weighed, sexed and examined for external, skeletal and soft tissue anomalies and developmental variations. The study was conducted according to the principles and practices of GLP (with QA certificate) and complied with OECD TG 414 (1981).

Analyses of the dosing preparations confirmed that the achieved concentrations, homogeneity and stability of cyromazine in the vehicle were satisfactory.

There were no treatment-related deaths during the study. Four females aborted and were sacrificed, one in the untreated control group, one in the group at 30 mg/kg bw per day and two in the group at 60 mg/kg bw per day (one on day 21 that may have been treatment-related). Decreased urination and defecation were noted in the groups at 30 and 60 mg/kg bw per day. At 60 mg/kg bw per day, severe maternal body-weight losses (−226 g on days 7–10, −113 g on days 7–20) and reduced food consumption (44% and 55% of vehicle control for days 7–10 and days 7–20, respectively) were seen, particularly over the first few days of the study. Mean body-weight gains and food consumption were significantly increased after the treatment period (days 20–29 of gestation: +306 g compared with +10 g in the vehicle control group and 169 g compared with 102 g in the vehicle control group, respectively). At 30 mg/kg bw per day, decreased body-weight gains during days 7–10, 10–14 and 7–20 of gestation were observed (17.5%, 87% and 69% of values for the vehicle control, respectively) as well as statistically significant decreases in food consumption during the first 3 days of treatment (82% of vehicle control). Treatment-related decreases in the mean net body-weight gain were observed in the groups at 30 and 60 mg/kg bw per day when compared with controls (days 0–29 of gestation, decrease of 6% and 14%, respectively). Mean gravid uterine weights and net maternal body weights were not affected by treatment at any dose. There were no pathological changes among the dams at the terminal necropsy which could be considered treatment-related. The pregnancy rate of the group at 10 mg/kg bw per day was somewhat lower than normally expected for this species (seven successful pregnancies out of 18 females inseminated). This was considered to be a random occurrence.

There were no treatment-related differences in the mean numbers of viable and dead fetuses, implantation sites and corpora lutea in the treated groups when compared with the vehicle and untreated

control groups. No significant differences in fetal sex ratios or fetal weights were observed in any of the treated groups. There was an increase (not statistically significant) in the mean number of early resorptions in the two groups at higher doses (mean, 1.2 and 1.7, respectively, compared with 0.8 and 0.9 in the controls) and the mean number of late resorptions in the group at the highest dose (mean, 0.6 compared with 0.1 and 0.0 in the controls). Mean postimplantation loss was increased in the group at 60 mg/kg bw per day compared with the controls (mean, 2.4 compared with 0.9 in the controls), which exceeded the incidence among historical controls (Buckshire: mean, 0.8; range, 0.7–0.9).

An increased number of fetuses with external malformations were seen in the group at 60 mg/kg bw per day compared with the control groups (Table 33). Four fetuses from one litter had open eyelid and three fetuses from another litter had shortened tails. The majority of the external malformations in the group at 30 mg/kg bw per day were observed in a fetus with multiple anomalies (spina bifida, exencephaly, cyclopia and umbilical hernia). The finding of cyclopia and related head malformations (exencephaly) in single fetuses in the groups at 10 and 30 mg/kg bw per day was not observed in the group at 60 mg/kg bw per day and was possibly of genetic origin as both fetuses were sired by the same male. Skeletal examination revealed there was a slight increase in the number of fetuses with vertebral anomalies (with associated rib anomaly) when compared with the control groups and data for historical controls. All visceral anomalies in the group at the highest dose occurred in single instances. In the group at 30 mg/kg bw per day, the percentage of visceral malformations was slightly increased in comparison with that in both control groups. In this group there were three fetuses with diaphragmatic hernia (in two litters, not observed in the group at the highest dose), two fetuses with hydrocephaly (incidence among historical controls, Buckshire: range for fetuses, 0.0–1.2; range for litters, 0.0–7.7) and one fetus with a kidney with an associated ureter anomaly. There was a statistically significant increase at 60 mg/kg bw per day in the incidence of 13th rudimentary ribs; however, the percentage of fetuses and litters affected was similar in frequency to the range for historical controls.

Table 33. Incidence of malformations of fetuses in a study of developmental toxicity in rabbits given cyromazine by gavage

Finding	No. of fetuses affected (No. of litters affected)					
	Dose (mg/kg per day)					
	0[a]		5	10	30	60
	0.5% CMC		Not treated			
Total No. examined	58 (12)	53 (13)	62 (12)	45° (7)	71 (10)	54 (11)
No. with external malformations	0 (0)	0 (0)	1 (1)	1 (1)	2 (2)	10 (5*)
Cyclopia with multiple head anomalies	0 (0)	0 (0)	0 (0)	1 (1)	1 (1)	0 (0)
No. with soft tissue malformations	1 (1)	0 (0)	1 (1)	2 (2)	6 (4)	3 (3)
No. with skeletal malformations	6 (6)	2 (2)	4 (2)	2 (1)	5 (5)	11 (6)
Vertebral anomaly with or without associated rib anomaly	4 (4)	1 (1)	3 (1)	1 (1)	3 (3)	7 (4)
Total number with malformations	7 (6)	2 (2)	5 (3)	3 (2)	10 (6)	15 (6)
Variations						
Thirteenth rudimentary ribs	2 (2)	4 (3)	5 (3)	3 (2)	7 (4)	10 (7*)

From Nemec (1985)

CMC, carboxymethyl cellulose

[a] Historical incidence of thirteenth rudimentary rib(s): 17/258 fetuses (range: 0.0–14.1%), 12/42 litters (range: 0.0–57.1%); and of vertebral anomalies: 8/258 fetuses (range: 0.0–4.7%), 7/42 litters (0.0–28.6%) (Buckshire)

* Statistically significant difference from control group mean, $p < 0.05$ (Fisher exact test); decreased number due to a decreased pregnancy rate attributed to random occurrence.

In New Zealand White rabbits, cyromazine produced severe maternal toxicity when administered orally at a dose of at 60 mg/kg bw per day during gestation, as shown by clinical signs of toxicity, one abortion, severe body-weight loss and decreased food consumption. Slight maternal toxicity (decreased body-weight gain and feed consumption) was also observed at 30 mg/kg bw per day. As a result of the severe maternal toxicity, postimplantation losses and resorptions increased at 60 mg/kg bw per day. At this dose, the number of fetuses (litters) with external malformations (not considered to be treatment-related), and the number of fetuses with vertebral anomalies were also increased. The NOAEL for maternal toxicity was 30 mg/kg bw per day considering that body-weight loss was marginal at that dose. The NOAEL for developmental toxicity was also 30 mg/kg bw per day (Nemec, 1985).

A study was undertaken in order to investigate the suspected deficiency in the male that sired two fetuses with a rare malformation, cyclopia, in two groups in the previous study. A total of four male New Zealand White (BUK:(CRL)NZWfBR) rabbits were used for artificial insemination, and sperm morphology and semen quality were evaluated. The male that had been used in the previous study of teratology was included to investigate whether this male would again produce fetuses showing cyclopia and/or related head effects. Three groups of at least 56 female rabbits were inseminated. Females in the 'sham' control group were 'sham' gavaged once daily from days 7 to 19 of gestation (a stainless-steel dosing cannula was used, but no material was administered). The females in the other two groups were not dosed. Clinical observations, body weights and food consumption were recorded throughout the study. On day 29 of gestation, all dams were killed and uterine contents examined. All fetuses were weighed, sexed and examined for external, skeletal and soft tissue anomalies and developmental variations. The study was conducted according to the principles and practices of GLP (with QA certificate).

Sixteen females died during the experimental period; 9, 5 and 2 from control group 1 (group size, 56), control group 2 (group size, 59) and the 'sham' control group (group size, 56), respectively. The cause of death for most of these animals was attributed to a suspected bacterial infection. There were no differences in clinical observations between the groups. A total of 43, 48 and 45 gravid females were available for examination at study termination from the control group 1, control group 2 and 'sham' control groups, respectively. There were no apparent differences between the groups in maternal body-weight gain or food consumption. There were no group differences in reproductive parameters.

Although cyclopia per se was not observed in this study, a number of rare and severe defects of the head and related structures occurred (Table 34). These included hydrocephaly, acrania, cleft palate (all observed in different fetuses) and one fetus with multiple head anomalies from male No. 2749. Defects of the head (hydrocephaly, acephaly and macroglossia) also occurred in fetuses sired by other males. In addition, other malformations observed in this study had been seen in the previous study of teratology. These included spina bifida, fused or severely malaligned sternebrae, hydrocephaly, short tail, heart and/or great vessel anomalies, vertebral defects, rib anomalies, mid-line closure defects, kidney anomalies and diaphragmatic hernia.

Table 34. Incidence of malformations in control groups of (BUK:(CRL)NZWfBR) New Zealand White rabbits

Type of malformation	No. of fetuses affected (No. of litters affected)[a]		
	Control group 1	Control group 2	Sham control group
Total No. examined	182 (33)	233 (39)	186 (37)
External malformations	1 (1)	6 (6)	7 (5)
Conjoined twins	0 (0)	1 (1)	0 (0)
Acephaly	0 (0)	1 (1)	0 (0)

Spina bifida	1 (1)	1 (1)	2 (2)
Short tail	0 (0)	1 (1)	1 (1)
Brachydactyly	0 (0)	0 (0)	1 (1)
Carpal and/or tarsal flexure	0 (0)	1 (1)	3 (3)
Gastroschisis	0 (0)	0 (0)	1 (1)
Acrania	0 (0)	0 (0)	1 (1)
Head anomaly*	0 (0)	0 (0)	1 (1)
Thoraco-gastroschisis	0 (0)	0 (0)	1 (1)
Cleft palate	0 (0)	0 (0)	1 (1)
Macroglossia	0 (0)	1 (1)	0 (0)
Soft tissue malformations	4 (4)	3 (3)	3 (3)
Retroesophageal aortic arch	0 (0)	1 (1)	0 (0)
Malpositioned kidneys	0 (0)	0 (0)	1 (1)
Heart and/or great vessel anomaly	1 (1)	0 (0)	0 (0)
Hydrocephaly	2 (2)	1 (1)	1 (1)
Diaphragmatic hernia	0 (0)	1 (1)	0 (0)
Interventricular septa defect	0 (0)	0 (0)	1 (1)
Kidney and/or ureter absent	0 (0)	1 (1)	0 (0)
Iris bombe	1 (1)	0 (0)	0 (0)
Skeletal malformations	10 (9)	10 (10)	11 (9)
Extra site of ossification anterior to sternebra No. 1	0 (0)	3 (3)	1 (1)
Sternebrae fused	2 (2)	2 (2)	5 (5)
Vertebral centra anomaly	2 (2)	2 (2)	1 (1)
Vertebral anomaly with or without associated rib anomaly	3 (2)	3 (3)	4 (3)
Rib anomaly	2 (2)	0 (0)	0 (0)
Sternebra(e) mal-aligned (severe)	2 (2)	1 (1)	1 (1)
Skull anomaly	0 (0)	1 (1)	0 (0)
Bent limb bone(s)	0 (0)	1 (1)	0 (0)
Total No. with malformations	15 (13)	15 (15)	16 (12)
Variations			
Thurteenth rudimentary ribs	30 (17)	27 (15)	21 (16)

[a] The number of litters affected is given in parentheses.

From Nemec (1986b)

In conclusion, the incidence of spontaneous fetal malformations in the BUK:(CRL)NZWfBR closed colony was found to be higher (6.4–8.6% for fetuses and 32.4–39.3% for litters) than that of the two other colonies of New Zealand White rabbits that had been used in the laboratory (Hazleton Research animals: 3.9% for fetuses and 21.5% for litters; Langshaw farms: 2.8% for fetuses and 15.6% for litters). The male from the previous study sired a number of fetuses with malformations in this study, including some with severe head anomalies. These results indicate that the malformations observed in the treated groups of the previous cyromazine study (Nemec, 1985) were consistent with those seen in this colony of rabbits (BUK:(CRL)NZWfBR). The effects of mild stress by sham gavage dosing on the incidence of malformations in this study were equivocal. Although an increase in the number of visceral malformations was associated with females that were sham gavaged, there were no remarkable overall differences observed between test groups with reference to the number of fetuses

and litters with malformations. The types of developmental variations were similar to those observed in the historical controls for the laboratory and in the study of Nemec, 1985 (Nemec, 1986b).

In a fourth study, groups of 74 artificially inseminated New Zealand White (Hra:(NZW)SPF) rabbits were given cyromazine (purity, 95.2%) at a dose of 0 (vehicle control, 0.5% aqueous CMC), 5, 10 or 30 mg/kg bw per day by gavage on days 7–19 (inclusive) of gestation. Throughout gestation, all females were observed twice per day for toxicity. Individual maternal body weights were recorded on days 0, 7, 10, 14, 20, 24 and 29 of gestation and food consumption was recorded daily from days 0 to 29 of gestation. On day 29 of gestation, 25 females with viable fetuses from each group were killed for teratological investigation. The uteri were examined for live fetuses and intrauterine deaths. The fetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination. The remaining females were allowed to litter naturally. On day 4 of lactation, six young per litter were randomly selected and retained for an assessment of postpartum survival and growth up to lactation day 28. Remaining young were killed after weighing on day 4. On day 28 of lactation, offspring were killed, sexed and necropsied. A gross postmortem examination was performed on all surviving F_0 maternal females. The study was conducted according to the principles and practices of GLP (with QA certificate) and complied with OECD TG 414 (1981).

Analyses of the dosing preparations confirmed that the achieved concentration, homogeneity and stability of the test substance in vehicle were satisfactory.

There was no evidence of any effect of cyromazine in the animals at 5 or 10 mg/kg bw per day, on clinical findings, survival, body-weight gain during gestation or lactation, food consumption or maternal performance. At 30 mg/kg bw per day, there was a marked body-weight loss in dams from days 7–20 of gestation (−115 g). After the dosing period, body-weight gain was statistically increased compared with that in the control group (+206 g and +70 g, respectively). Body-weight gain during lactation in these females was generally comparable to that of controls. Food consumption in the group at 30 mg/kg bw per day was affected in a corresponding manner (days 10–14, 67% and days 14–20, 65% of values for controls).

The mean numbers of viable and nonviable fetuses, early and late resorption sites, postimplantation loss, implantation sites, corpora lutea and mean fetal weights were similar in treated and control groups.

There were no biologically meaningful or statistically significant differences in the number or percentage of fetuses with malformations or developmental variations in any of the cyromazine-treated groups compared with controls. Postnatally, there were no treatment-related alterations in duration of gestation, parturition, live birth and survival indices, sex ratio, live litter size, offspring body weight or condition throughout lactation.

In conclusion, cyromazine produced maternal toxicity (body-weight loss and decreased food consumption) at 30 mg/kg bw per day when administered orally to New Zealand White rabbits during the period of major organogenesis. The NOAEL for maternal toxicity was 10 mg/kg bw per day. Cyromazine did not induce teratogenic or fetotoxic effects at doses up to 30 mg/kg bw per day, the highest dose tested in this study (Nemec, 1986a).

2.6 Special studies: neurotoxicity

Cyromazine belongs to the chemical class of s-triazines; it acts as an insect growth regulator and is not suspected to act upon the nervous system. Except for some unspecific symptoms at doses at or above the LD_{50} observed in studies of acute toxicity , the acute, short- and long-term studies reported previously revealed neither clinical signs nor any biochemical or histopathological change that might point to a neurotoxic potential of cyromazine. The conduct of special studies in the field of neurotoxicity was therefore not necessary.

3. Studies on metabolites

There were no metabolites found to a significant amount in plants and/or soil that were not seen in animals. In soil, considering the degradation and mineralization, it was concluded that the risk of cyromazine or one of its metabolites being translocated into non-target areas, including groundwater, was low. The predicted environmental concentration (PEC) at 1 m soil depth, after use of products that contain cyromazine under realistic worst-case scenarios was less than 0.00 µg/l. If melamine occurs as a metabolite in groundwater, it is also predicted not to exceed 0.001 µg/l for most European soil and climate scenarios under common conditions of agricultural use.

Melamine is a metabolite in rats (about 7% in urine and faeces), monkeys, goats and hens. It is an important chemical commodity used for the production of melamine–formaldehyde resins. The toxicity of melamine has been extensively investigated and reported in the literature. Reviews had been released by ECETOC (1983) and IARC (1986 & 1999). Melamine has no insecticidal activity in comparison with the parent cyromazine (Rindlisbacher, 2002). In rats and humans, melamine is a metabolite of the anti-neoplastic agent hexamethylmelamine (Worzalla et al., 1974). The carcinogenic potency of melamine had been investigated in the National Toxicology Program (1983). The results are discussed below.

3.1 Absorption, distribution, excretion and metabolism

After administration of melamine as a single oral dose at 250 mg/kg bw in rats or dogs, 50% and 61.3%, respectively, of unmodified melamine was excreted in the urine in 6 h (Lipschitz & Stokey, 1945). Nearly 20% of the melamine excreted by the rats was recovered as the crystalline dimelamine-monophosphate.

In adult male Fischer 344/N rats given [^{14}C]melamine as a single oral dose at 0.38 mg, melamine was rapidly excreted (90% of the administered dose within 24 h in the urine). Negligible radioactivity was detected in exhaled air and faeces. Melamine was distributed in the body water. Kidney and bladder showed higher concentrations of radioactivity than did plasma. Virtually no residual radioactivity was observed in the tissues at 24 h after dosing or later. The plasma half-life was 2.7 h, which is in good agreement with the urinary excretion half-life of 3.0 h. Radioactivity in plasma and urine co-chromatographed with unchanged melamine, indicating that melamine is not metabolized in the rat (Mast et al., 1983).

3.2 Toxicological studies

(a) Acute toxicity

Melamine has very low acute toxicity. The oral LD_{50} values for melamine given in corn oil by gavage were reported to be 3161 and 3828 mg/kg bw in male and female Fischer 344/N rats, and 3296 and 7014 mg/kg bw for male and female B6C3F_1 mice (National Toxicology Program, 1983). White crystals were found in the stomach of three out of five male and four out of five female rats at 10 000 mg/kg bw, four out of five males and five out of five females at 6810 mg/kg bw, one out of five males and two out of five females at 3160 mg/kg bw and one out of five males at 2150 mg/kg bw. In mice, no compound-related toxic effects were observed at necropsy. In another study in mice, the oral LD_{50} was 4500 mg/kg bw. Reported signs of toxicity were lachrimation, dyspnoea, intermittent tremors and coma preceding death. Vasodilatation in the tail and ears and paralysis of the forequarters were also observed (ECETOC, 1983). Melamine applied as a paste to the skin of rabbits at 1000 mg/kg bw did not induce toxicity or local irritation (ECETOC, 1983). Introduction

of melamine powder into the rabbit eye caused mild transient irritation (ECETOC, 1983). There was no sensitization in guinea-pigs (ECETOC, 1983).

(b) Short-term studies of toxicity

Mice

Groups of five male and five female B6C3F$_1$ mice were fed diets containing melamine (purity, 97%) at a concentration of 5000, 10 000, 15 000, 20 000, or 30 000 ppm for 14 days. All animals survived to the end of the study. Treatment seemed to have no effect on body weight. A hard crystalline solid was found in the urinary bladder of all male mice and in two out of five female mice at 30 000 ppm. No other compound-related effects were observed at necropsy (National Toxicology Program, 1983).

Groups of 10 male and 10 female B6C3F$_1$ mice were fed diets containing melamine at a concentration of 0, 6000, 9000, 12 000, 15 000, or 18 000 ppm (equivalent to 0, 900, 1350, 1800, 2250 and 2700 mg/kg bw per day) for 13 weeks. One female at 9000 ppm died. Body-weight gain was depressed in all treated groups. The incidence of mice with bladder calculi was dose-related and was greater in males than in females. Bladder calculi were found at dietary concentrations of 12 000 ppm and greater (6 out of 10, 9 out of 10 and 7 out of 10 in males, and 1 out of 10, 3 out of 10 and 7 out of 10 in females, at 12 000, 15 000 and 18 000 ppm, respectively). Ulceration of the urinary bladder epithelium was also dose-related and was observed at dietary concentrations of 9000 ppm and greater. Bladder ulcers were multifocal or associated with inflammation (cystitis). The results were considered not to provide evidence for an association between ulceration and bladder calculi in either sex. Since the body weight effects at 6000 ppm (equivalent to 900 mg/kg bw per day) were of questionable biological significance, this dose could be considered as a NOAEL (National Toxicology Program, 1983).

Rats

In rats treated with melamine, no histological effects were seen, except for crystalline deposits in the renal tubules found after five successive intraperitoneal doses at 500 mg/kg bw per day. No symptoms were observed except for moderate transient weight loss (ECETOC, 1983).

Groups of five male and five female Fischer 344/N rats were fed diets containing melamine (purity, 97%) at a concentration of 5000, 10 000, 15 000, 20 000, or 30 000 ppm for 14 days. Body-weight gain was reduced at dietary concentrations of 15 000 ppm and greater. A hard crystalline solid was found in the urinary bladder of most males at ≥ 10 000 ppm and in four out of five females at ≥ 20 000 ppm. The kidneys of two out of five males at 30 000 ppm were pale and pitted. The details given of the study were limited, but the highest NOAEL appeared to be 5000 ppm (National Toxicology Program, 1983).

Three 13-week studies were performed in rats.

In the first study, groups of 12 male and 12 female F344/N rats were fed diets containing melamine (purity, 97%) at a concentration of 0, 6000, 9000, 12 000, 15 000, or 18 000 ppm, equivalent to 0, 600, 900, 1200, 1500 and 1800 mg/kg bw per day. One male at 18 000 ppm and two males at 6000 ppm died. Body-weight gain at a dietary concentration of 12 000 ppm and greater was depressed and food consumption was reduced at 18 000 ppm. Calculi were found to occur in the urinary bladder

of most male rats in a dose-related manner and in the bladder of some females at ≥ 15 000 ppm. Histopathological evaluation was performed on 10 animals of each sex from the control group and groups at, 6000 and 18 000 ppm. Diffuse epithelial hyperplasia of the urinary bladder was found in 8 out of 10 males and 2 out of 10 females at 18 000 ppm, while in rats at 6000 ppm, focal epithelial hyperplasia was only found in 1 out of 10 males and in none of the females. No other compound-related histopathological effects were observed (National Toxicology Program, 1983).

In the second 13-week study, groups of male and female F344/N rats were fed diets containing melamine (purity, 97%) at a concentration of 0, 750, 1500, 3000, 6000, or 12 000 ppm, equivalent to 0, 75, 150, 300, 600 and 1200 mg/kg bw per day. Body-weight gain was depressed in males only at 6000 and 12 000 ppm. Urinary bladder calculi were not observed in treated or control females, but the incidence among male rats increased in a dose-related manner (1 out of 10, 2 out of 10, 5 out of 10, 7 out of 10, 9 out of 10 and 9 out of 9 in the controls and at 750, 1500, 3000, 6000 and 12 000 ppm, respectively). Hyperplasia of the transitional epithelium of the bladder was observed to occur in a dose-dependent manner for male rats at ≥ 3000 ppm (1 out of 10, 3 out of 10 and 9 out of 9in the groups at 3000, 6000 and 12 000 ppm, respectively). There was no evidence of hyperplasia of the bladder epithelium in female rats. Dose-related calcareous deposits were observed in the straight segments of the proximal tubules in females. Microscopic evaluation of the urine did not provide evidence of melamine crystalluria. The NOAEL for hyperplasia was 1500 ppm, equivalent to 150 mg/kg bw per day (National Toxicology Program, 1983).

In the third 13-week study, F344/N rats were fed diets containing melamine (purity, 97%) at a concentration of 0, 10 000 or 18 000 ppm in the presence and absence of 1% ammonium chloride in the drinking-water to see if this treatment might affect the incidence of calculus formation in the urinary tract. Ammonium chloride had no effect on calculus formation (National Toxicology Program, 1983).

Rabbits and dogs

In rabbits and dogs fed with melamine at a dose of 126 mg/kg bw per day for 1 to 4 weeks, no effects were found, either macro- or microscopically (ECETOC, 1983).

(c) Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female B6C3F_1 mice were fed diets containing melamine (purity, 97%) at a concentration of 0, 2250 or 4500 ppm (equivalent to 0, 338 or 675 mg/kg bw per day) for 103 weeks, followed by a basal diet for 2 weeks before sacrifice. All animals were observed twice daily for morbidity or mortality. Clinical signs were recorded monthly. Body weight and feed consumption by cage (five animals per cage) were recorded once per week for the first 13 weeks, monthly until week 91 and then every 2 weeks. Necropsies were performed on all animals found dead and those killed at the end of the study. Animals were examined for gross and histopathological abnormalities.

Mean body weights of male mice at the highest dose were slightly lower than those of controls after week 50 of the study (decrease in body-weight gain, 21%). Survival at termination of the study was: 39 out of 49 (80%), 36 out of 50 (72%) and 28 out of 50 (56%) in males in the control group and at the lower and higher dose, respectively and 37 out of 50 (74%), 43 out of 50 (86%) and 41 out of 50 (82%) in females in the control group and at the lower and higher dose, respectively. The

reduction in the survival of males at the highest dose was statistically significant ($p = 0.013$). No treatment-related increase in the incidence of tumours was observed. In male mice, treatment-related increases were observed in the incidence of urinary bladder calculi (2 out of 45, 40 out of 47 and 41 out of 44 in the control group and at the lower and higher dose, respectively), in the incidence of acute and chronic inflammation of the urinary bladder (0 out of 45, 25 out of 47 and 24 out of 44 in mice in the control group and at the lower and higher dose, respectively) and in the incidence of epithelial hyperplasia of the bladder (1 out of 45, 11 out of 47 and 13 out of 44 in mice in the control group and at the lower and higher dose, resectively). Urinary bladder calculi, acute and chronic inflammation of the urinary bladder and epithelial hyperplasia were seen in 4 out of 50 females at the highest dose (National Toxicology Program, 1983).

Rats

Groups of 50 male and 50 female Fischer 344/N rats were fed diets containing melamine at a concentration of 0, 2250 or 4500 ppm (males) or 0, 4500 or 9000 ppm (females) for 103 weeks, followed by a basal diet for 2 weeks before sacrifice. Doses were equivalent to 0, 113 or 225 in males and 0, 225 or 450 mg/kg bw per day in females. All animals were observed twice daily for morbidity or mortality. Clinical signs were recorded monthly. Body weight and feed consumption by cage (five animals per cage) were recorded once per week for the first 13 weeks, monthly until week 91 and then every 2 weeks. Necropsies were performed on all animals found dead and those killed at the end of the study. Animals were examined for gross and histopathological abnormalities.

Survival was significantly reduced in males at the highest dose ($p = 0.03$) from week 101. Survival rates at termination of the study were: 30 out of 49 (61%), 30 out of 50 (60%) and 19 out of 50 (38%) in males in the control group and at the lowest and highest dose, respectively and 34 out of 50 (68%), 30 out of 50 (60%) and 27 out of 50 (54%) in females in the control group and at the lower and higher dose, respectively. The incidence of transitional-cell carcinomas of the urinary bladder in males was 0 out of 45, 0 out of 50 and 8 out of 49 (trend $p \leq 0.002$) in the control group and the lower and higher dose, respectively. The incidence in the group at the highest dose was significantly higher ($p \leq 0.016$) than in the controls. There was also a dose-related incidence of bladder calculi in males (0 out of 45, 1 out of 50, and 10 out of 49 in the control group and at the lower and higher dose, respectively). In a separate study, X-ray microscopic analysis of two urinary bladder calculi obtained from male F344/N rats fed diets containing melamine at 16 000 or 19 000 ppm indicated that the principal component of the calculi was melamine. Of 49 males at the highest dose, seven had transitional-cell carcinomas and bladder calculi, one had a carcinoma without calculi and three had calculi without carcinoma (one of these rats had a papilloma and one had epithelial hyperplasia). There was therefore a statistically significant ($p < 0.001$) correlation between the presence of bladder calculi and bladder tumours. Females had no bladder calculi and one female in each of the groups at the lowest and highest dose had a papilloma of the bladder. A statistically significant increase ($p \leq 0.01$) in the incidence of chronic inflammation of the kidney was observed in females (4 out of 49, 17 out of 49 and 41 out of 47 in the control group and at the lower and higher dose, respectively). The dose–response relationship and intensity of the increased interstitial lymphoplasmocytic infiltrates and cortical fibrosis clearly set these changes apart from the minor inflammatory component that may accompany the progressive nephropathy normally encountered in ageing F344/N rats. Chronic inflammation of the kidney was not significant in male rats receiving cyromazine (National Toxicology Program, 1983).

Groups of 20 male F344 rats were fed diets containing melamine (purity, > 99%) at a concentration of 0.3%, 1% or 3% (equivalent to 150, 500 and 1500 mg/kg bw per day) for a total of 36 weeks followed by a 4-week recovery period. Ten animals per group underwent exploratory laparotomy at the end of week 36 and all animals were killed at week 40. Bladder weight in rats

receiving 3% melamine in the diet was threefold that in controls. The incidences of papillary or nodular hyperplasia were 0%, 5%, 30% and 63% in the control group and at the lowest, intermediate and highest dose, respectively; incidences of papillomatosis were 0%, 0%, 25% and 89% and those of calculi were 0%, 0%, 70% and 100% at 36 weeks and 0%, 20%, 45% and 42% at 40 weeks. Carcinomas of the urinary bladder were observed in 0 out of 20, 1 out of 20 and 15 out of 19 rats at the lowest, intermediate and highest doses and papillomas in 0 out of 20, 1 out of 20 and 12 out of 19 rats, respectively. One carcinoma and three papillomas of the ureter were also induced in 19 rats at the highest dose. The correlation between calculus formation at week 36 and tumour incidence at week 40 was highly significant ($p = 0.0065$) (Okumura et al., 1992; IARC, 1999).

Groups of 20 male F344/DuCrj rats were fed diets containing melamine (purity, 99.94%) at a concentration of 1% or 3% with or without 5% or 10% aqueous sodium chloride solution (NaCl) for a total of 36 weeks and were killed at week 40. Water intake, as a surrogate for urinary output, was increased in groups exposed to 3% melamine, with or without 5% or 10% NaCl, in groups exposed to 1% melamine with 5% or 10% NaCl and in a group exposed to 10% NaCl only; water intake was not increased in animals exposed to 1% melamine only. The incidences of calculi and papillomatosis were 30% and 75% with 3% melamine, 75% and 85% with 3% melamine plus 5% NaCl, 30% and 10% with 3% melamine plus 10% NaCl, 37% and 47% with 1% melamine, 11% and 11% with 1% melamine plus 5% NaCl, 5% and 0% with 1% melamine plus 10% NaCl. No calculi or papillomatosis were reported in controls or with 10% NaCl alone.

Urinary bladder carcinomas were observed in 4 out of 19, 18 out of 20 and 18 out of 20 rats given 1% melamine alone, 3% melamine alone or 3% melamine plus 5% NaCl, respectively. No carcinomas were observed in the groups receiving 3% melamine plus 10% NaCl or 1% melamine plus 5% or 10% NaCl. The incidences of papillomas were similarly decreased by NaCl. In contrast to the incidence of 10 out of 20 in the group given 3% melamine alone, 5 out of 20 and 3 out of 20 rats receiving 3% melamine plus 5% NaCl or 10% NaCl respectively, developed papillomas. Papillomas developed in 8 out of 19 rats receiving 1% melamine alone.

Therefore, the addition of NaCl to 1% melamine decreased the incidences of calculi and papillomatosis, in parallel with a decrease in the incidence of neoplasia. With 3% melamine, NaCl did not affect the induction of calculi or papillomatosis but decreased the incidence of neoplasia. Thus, with the lower concentration of melamine, NaCl appeared to increase urinary output and decrease the incidences of hyperplasia, calculus formation and neoplasia. Chemical analysis of the calculi showed that they contained approximately equal amounts of melamine and uric acid on a molar basis, which together accounted for 61–81% of the weight (Ogasawara et al., 1995; IARC, 1999).

On the basis of these studies, it has been concluded that bladder tumours are associated with administration of high doses of melamine, and that the tumours are related to precipitation of urinary melamine with the formation of melamine/uric acid containing urinary-tract calculi, producing urothelial toxicity and consequent regeneration of the bladder epithelium and ultimately the formation of tumours (IARC, 1999; Meek et al., 2003). Although the correlation between calculi, ulceration, hyperplasia and formation of bladder tumours has not been 100%, explanations on the basis of studies with other chemicals such as uracil have been advanced to explain the discrepancies (Clayson et al., 1995, Fukushima et al., 1992). Ulcerations secondary to calculi formation occur relatively rapidly and are repaired, even with continued presence of the calculus. It is thus not unusual to see extensive proliferation of the bladder epithelium in the presence of calculi at later time-points, such as those seen in the experiments with melamine, without an associated ulceration or intense inflammatory response. Chronic inflammation is frequently present, however. Similarly, correlation between the presence of calculi and tumours at later time-points is not 100%. This has been explained by the loss of calculi during the experiment, either by dissolution or, more likely, spontaneous evacuation from the urinary tract (Clayson et al., 1995; IARC, 1999b).

There is significantly less risk in humans for developing bladder cancer from calculi than in rodents, most likely owing primarily to the usually short time that calculi are present in humans due to anatomic and obstructive issues (IARC, 1999b).

(d) Genotoxicity

The genotoxic potential of melamine was assessed in several tests in vitro and in vivo (Table 35). With the exception of prophage induction, all the tests gave negative results. is the Meeting considered that melamine is not genotoxic.

Table 35. Results of studies of genotoxicity with melamine, a metabolite of cyromazine

End-point	Test object	Concentration	Results	Reference
Prophage induction	*E. coli* WP2s (λ)	78 µg/well	Positive[a]	Rossman et al. (1991)
Reverse mutation	*S. typhimurium his* G46, TA1530, TA1531, TA1532 and TA1534	Not stated	Negative[c]	Seiler (1973)
	S. typhimurium TA1535, TA1537, TA1538, TA98 and TA100 *E. coli* WP2 *uvrA*	≤ 500 µg/plate	Negative[a]	Jagannath & Brusick (1977)
	S. typhimurium TA1535, TA1538, TA98 and TA100	Not stated	Negative	Lusby et al. (1979)
	S. typhimurium TA1535, TA1537, TA1538, TA98 and TA100	≤ 5000 µg/plate, plate incorporation assay	Negative[a]	Mast et al. (1982a)
	S. typhimurium TA1535, TA1537, TA98 and TA100	≤ 5550 µg/plate, pre-incubation assay	Negative[a,b]	Haworth et al. (1983) National Toxicology Program (1983)
Gene conversion	*S. cerevisiae* D4	Not stated	Negative[a]	Jagannath & Brusick (1977)
Forward mutation	Chinese hamster ovary (CHO), *Hprt* locus	0.6–1 mg/ml	Negative[a]	Mast et al. (1982a) National Toxicology Program (1983)
Gene mutation	Mouse lymphoma L5178Y, *Tk* locus	≤ 160 µg/ml	Negative[a]	McGregor et al. (1988)
Chromosomal aberration	CHO	Not stated	Negative	National Toxicology Program (1983)
Sister chromatid exchange	CHO	≤ 1 mg/ml	Negative[a]	Mast et al. (1982a)
Unscheduled DNA synthesis	Primary rat hepatocytes	≤ 6 mg/ml	Negative	Mirsalis et al. (1983); National Toxicology Program (1983); ECETOC (1983)
Sex-linked recessive lethal mutations	*Drosophila melanogaster*	1% feed	Negative	Röhrborn (1962)
Micronucleus test (BM cells)	Mice	Single oral dose at 1000 mg/kg bw or 2 × 1000 mg/kg bw (24 h apart)	Negative	Mast et al. (1982b) National Toxicology Program (1983)

S9, 9000 × *g* supernatant from livers of Aroclor-induced rats or hamsters.

[a] With and without metabolic activation.

[b] S9 from the liver of Aroclor-induced rats or hamsters, liquid pre-incubation assay.

[c] Without S9.

(e) Reproductive toxicity

No toxic effect or gross malformation was found in fetuses of pregnant rats injected intraperitoneally with melamine at a dose of 70 mg/kg bw on days 5 and 6, 8 and 9 or 12 and 13 of gestation (Thiersch, 1957). This study was considered to be inadequate for an evaluation of prenatal toxicity owing to incomplete reporting of experimental methods and results of fetal examinations (IARC, 1999).

(f) Special studies

A study of skin initiation/promotion to investigate the initiation activity of melamine was performed in CD-1 mice. At a single topical dose of 1 μmol (approx. 6 mg/kg bw) melamine (unknown purity) in 0.2 ml acetone followed by twice-weekly applications of 10 nmol of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 0.2 ml acetone for 31 weeks, there was no increase in the incidence of papillomas in comparison to controls (acetone + TPA) (19% compared with 14% in controls) (Perrella & Boutwell, 1983; IARC, 1986).

4. Observations in humans

Routine medical examinations, including anamnesis, physical examination and comprehensive analysis of blood and urine from employees who had handled cyromazine in different manufacturing or formulating plants had revealed no adverse effects on health and no complaints had been reported (SYNGENTA, medical data from manufacturing plants, Monthey, Switzerland, and Grimsby, UK, 1994).

No cases of poisoning of workers involved in the production and formulation or field use of cyromazine had been reported to the company or in the open literature. No epidemiological study was available.

Comments

Biochemical aspects

Toxicokinetic studies in rats given ^{14}C-labelled cyromazine as single or repeated oral doses showed that the active substance is rapidly and almost completely absorbed from the gastrointestinal tract and distributed to all organs and tissues. The substance was rapidly excreted, with an initial rapid phase of 2–12 h followed by a slower phase. More than 97% of the administered dose was excreted within 24 h, almost exclusively in the urine.

Cyromazine was incompletely metabolized, essentially by methylation, hydroxylation or *N*-dealkylation. The major component present was cyromazine, which accounted for 71–72% of the radiolabel; a further 7% was attributable to melamine, 8–11% to hydroxy-cyromazine and methyl-cyromazine. Only 6% of [U-^{14}C triazine]metabolites in the urine and 13% in the faeces remained uncharacterized and comprised minor metabolites.

In monkeys (*Macaca fasicicula*), ^{14}C-labelled cyromazine was also rapidly and extensively absorbed and rapidly excreted, predominantly in the urine. Cyromazine accounted for approximately 95% of urinary radioactivity, with the remainder being attributable to melamine.

Toxicological data

Cyromazine has low acute toxicity in rats when administered orally (LD_{50} = 3387 mg/kg bw), dermally (LD_{50} > 3170 mg/kg bw) or by inhalation (4-h LC_{50} > 3.6 mg/l, the highest achievable concentration). Signs of intoxication were sedation, dyspnoea, curved position and ruffled fur after oral or dermal administration. Animals recovered from systemic symptoms within 9–12 days. After inhalation, a decrease in activity, piloerection and nasal discharge were observed; these clinical signs were no longer seen on day 2 after exposure. Cyromazine is not an irritant to the skin and eyes of rabbits. In a maximization test in guinea-pigs, cyromazine did not show any sensitizing potential.

The toxicity of cyromazine administered orally was investigated in short-term dietary studies: 90-day studies in rats and dogs and a 1-year study in dogs. The main effects were changes in body weight in rats and dogs, and haematological changes in dogs.

The NOAEL in a 90-day study in rats was 3000 ppm, equal to 232 mg/kg bw per day, the highest dose tested. Small changes in body weight were not considered to be toxicologically relevant.

In a 90-day study in dogs, cyromazine induced some reduction in body-weight gain in both sexes at 3000 ppm and in females at 1000 ppm. Food consumption was decreased at 3000 ppm. Decreased erythrocyte values (total erythrocyte count, haemoglobin concentration and erythrocyte volume fraction) were observed in males and females at 3000 ppm and 300 ppm, respectively. The NOAEL was 300 ppm (equal to 12 mg/kg bw per day).

In a 1-year dietary study in dogs, the NOAEL was 200 ppm, equal to 5.7 mg/kg bw per day, on the basis of haematological effects observed at 800 ppm in males.

A NOAEC of 0.058 mg/l, equivalent to 9.3 mg/kg bw per day, was identified on the basis of clinical signs in a 28-day (4 h per day) study in rats treated by inhalation. Haematology was reversibly affected in males at 0.706 mg/l. In rabbits, dermal exposure to cyromazine at doses of up to 2000 mg/kg bw per day for 21 days (6 h per day) produced no systemic adverse effects and no observable skin irritation.

Long-term dietary studies of toxicity and carcinogenicity were carried out in mice and rats. Body-weight changes were the critical effects observed in these studies.

The NOAEL in a 2-year study in mice given diets containing cyromazine was 1000 ppm (equal to 126 mg/kg bw per day) on the basis of changes in body weight in males at 3000 ppm. Small and occasional decreases in body weight and food consumption observed at 1000 ppm were not considered toxicologically relevant.

In rats, dietary administration of cyromazine for 2 years resulted in a decrease in mean body weight, body-weight gain and food consumption in males and females at 3000 ppm. The NOAEL for these effects was 300 ppm, equal to 15 mg/kg bw per day.

Non-statistically significant increases in the incidence of mammary gland tumours were observed in female mice (above the highest value in the range for historical controls) and rats (within the range for historical controls) at 3000 ppm.

Cyromazine gave consistently negative results in a comprehensive range of studies of genotoxicity in vitro and in vivo, with the exception of an inconclusive spot test in mice. The Meeting concluded that cyromazine is unlikely to be genotoxic.

In view of the absence of genotoxicity and the equivocal response at the highest dose in the studies of carcinogenicity in mice and rats, the Meeting concluded that cyromazine is unlikely to pose a carcinogenic risk to humans at exposure levels relevant to residues on food.

The reproductive toxicity of cyromazine was examined in a two-generation study in rats, and in studies of developmental toxicity in rats and rabbits.

Dietary administration of cyromazine to rats for two generations resulted in decreased parental body weights and food consumption at doses of 3000 ppm, and decreased pup body weights at 3000 ppm. Male fertility was reduced in the F_0 generation at 3000 ppm. A decrease in pup viability at birth and in mean litter size was observed in F_2 litters at 3000 ppm. The NOAEL for parental toxicity, reproductive and offspring toxicity was 1000 ppm (equal to 51 mg/kg bw per day).

Cyromazine was not teratogenic to rats when administered at a dose of up to 600 mg/kg bw per day. Signs of maternal toxicity were observed at 300 and 600 mg/kg bw per day and fetal toxicity was observed at the highest dose. The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of clinical signs of toxicity and decreased body-weight gain. The NOAEL for developmental toxicity was 300 mg/kg bw per day on the basis of a decrease in body weight and reduced ossification at the next highest dose.

In three studies of developmental toxicity in rabbits, the administration of cyromazine by gavage at a dose of 25 mg/kg bw per day or greater resulted in deaths, clinical signs of toxicity (decreased urination and defecation), decrease in body-weight gain, body-weight loss and decrease in food consumption in the dams. Body-weight loss was usually observed within the first few days after dosing and it was not always possible to determine whether this loss was accompanied by a reduction in food intake. The animals rapidly recovered weight after dosing stopped. The overall NOAEL for maternal toxicity was 10 mg/kg bw per day. Cyromazine did not induce teratogenic or fetotoxic effects in rabbits. Increased numbers of abortions, post-implantation losses, resorptions and vertebral variations as well as decreased numbers of viable fetuses were observed only at maternally toxic doses (60 mg/kg bw per day or greater). The overall NOAEL for developmental toxicity was 30 mg/kg bw per day.

No specific studies of neurotoxicity with cyromazine were available; however, no evidence of neurotoxicity was apparent in the available studies of toxicity.

No adverse effects were reported in personnel involved in the production and formulation of cyromazine, or in the use of this product in the field.

The metabolites found in plants, goats, hens and rats are melamine and 1-methylcyromazine; neither contain new functional groups or structural alerts. Melamine has been investigated for its toxicological properties and results were reported in the published literature. The main toxic effects of dietary exposure to melamine in rats and mice were calculi formation (constituted by melamine and uric acid), inflammatory reactions and hyperplasia in the urinary bladder. The NOAEL for urinary bladder calculi formation and hyperplasia was 1500 ppm (equivalent to 150 mg/kg bw per day) in a 90-day study in rats. Induction of carcinomas of the urinary bladder occurred in male rats fed diets containing melamine at 4500 ppm (equivalent to 225 mg/kg bw per day) for 103 weeks, but not in female rats or in male or female mice. Melamine is not genotoxic in vitro or in vivo. Although bladder tumours related to calculi formation are not considered to be species-specific, they are related to the administration of high doses (IARC, 1999b). Bladder tumours were related to precipitation of urinary melamine with the formation of melamine/uric acid-containing urinary-tract calculi, producing urothelial toxicity and consequent regeneration of the bladder epithelium and ultimately formation of tumours. The non-DNA-reactive mechanism by which melamine produced urinary bladder tumours in male rats occurred only under conditions in which calculi were produced. The risk of developing bladder cancer from calculi is significantly lower in humans than in rodents, most probably because of the usually short time that calculi are present in humans, owing to anatomic and obstructive issues. These bladder tumours are thus an effect that occurs at high doses, having a threshold that is well above the expected human exposure through residues.

The Meeting concluded that the existing database was adequate to characterize the potential hazard of cyromazine and its metabolites to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.06 mg/kg bw based on a NOAEL of 5.7 mg/kg bw per day for haematological effects detected at 23 mg/kg bw per day in males in a 1-year study of toxicity in dogs, and using a safety factor of 100.

The Meeting established an ARfD of 0.1 mg/kg bw based on a NOAEL of 10 mg/kg bw per day for body-weight loss and decrease in food consumption observed soon after dosing at 25 mg/kg bw per day or greater in dams in studies of developmental toxicity in rabbits treated by gavage and with a safety factor of 100. The reason for these effects was unknown and there is a rapid recovery on cessation of administration. Therefore, this ARfD may be conservative.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	1000 ppm, equal to 126 mg/kg bw per day	3000 ppm, equal to 384 mg/kg bw per day
		Carcinogenicity	1000 ppm, equal to 164 mg/kg bw per day	3000 ppm, equal to 476 mg/kg bw per day
Rat	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	300 ppm, equal to 15 mg/kg bw per day	3000 ppm, equal to 156 mg/kg bw per day
		Carcinogenicity	3000 ppm, equal to 156 mg/kg bw per day[c]	—
	Multigeneration reproductive toxicity[a]	Parental	1000 ppm, equal to 51 mg/kg bw per day	3000 ppm, equal to 169 mg/kg bw per day
		Offspring toxicity	1000 ppm, equal to 51 mg/kg bw per day	3000 ppm, equal to 169 mg/kg bw per day
		Reproductive toxicity	1000 ppm, equal to 51 mg/kg bw per day	3000 ppm, equal to 169 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Developmental toxicity	300 mg/kg bw per day	600 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	25 mg/kg bw per day
		Developmental toxicity	30 mg/kg bw per day	60 mg/kg bw per day
Dog	One-year study of toxicity[a]	Toxicity	200 ppm, equal to 5.7 mg/kg bw per day	800 ppm, equal to 23 mg/kg bw per day

[a] Dietary administration

[b] Gavage administration

[c] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.06 mg/kg bw

Estimate of acute reference dose

0.1 mg/kg bw

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 94–97% based on urinary excretion
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid and extensive (> 97% within 24 h, mainly via urine)
Metabolism in animals	Incomplete metabolism, essentially by methylation, hydroxylation or *N*-dealkylation
Toxicologically significant compounds	Parent compound and melamine
Acute toxicity	
Rat, LD_{50}, oral	3387 mg/kg bw
Rat, LD_{50}, dermal	> 3170 mg/kg bw
Rat, LC_{50}, inhalation	> 3.6 mg/l of air (4-h, nose only, aerosol)
Rabbit, skin irritation	Not irritating (24 h)
Rabbit, eye irritation	Not irritating
Guinea-pig, skin sensitization (test method used)	Not sensitizing (Magnusson & Kligman)
Short-term studies of toxicity	
Target/critical effect	Decreased body-weight gain (rats and dogs), haematopoietic system (dogs)
Lowest relevant oral NOAEL	232 mg/kg bw per day (90-day study in rats) 5.7 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	2000 mg/kg bw per day, highest dose tested (3-week study in rabbits)
Lowest relevant inhalation NOAEC	0.058 mg/l air (28-day study in rats)
Genotoxicity	
	Not genotoxic in vitro and in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Decreased body-weight gain (mice and rats)
Lowest relevant NOAEL	15 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Non-statistically significant increase in incidence of mammary gland tumours in female mice (higher than historical control range) and rats at 3000 ppm
Reproductive toxicity	
Reproduction target/critical effect	Reduction in male fertility, decreased pup viability at birth and mean litter size at parentally toxic levels
Lowest relevant reproductive NOAEL	Parents: 51 mg/kg bw per day (rats) Reproductive and offspring toxicity: 51 mg/kg bw per day (rats)
Developmental target/critical effect	Embryotoxicity (rabbits) and fetotoxicity (rats) at maternally toxic doses
Lowest relevant developmental NOAEL	Maternal: 10 mg/kg bw per day (rabbits) Developmental: 30 mg/kg bw per day (rabbits)
Neurotoxicity/delayed neurotoxicity	
	No specific study; no findings in other studies

Other toxicological studies	
Toxicity of metabolites:Melamine	Oral LD_{50} in rat: 3161 mg/kg bw (males)
	Main toxic effects of dietary administration to rats and mice: calculi formation, inflammatory reactions and hyperplasia in the urinary bladder
	LOAEL calculus formation: about 150 mg/kg bw per day in rats (90 days)
	NOAEL hyperplasia: 150 mg/kg bw per day in rats (90 days) Induction of carcinomas of the urinary bladder in male rats at 225 mg/kg bw per day (2 years)
	Not carcinogenic in female rats or in mice of both sexesNot genotoxic in vitro and in vivo
	Non-genotoxic mode of action
Medical data	
	No adverse effects on health in manufacturing personnel

Summary

	Value	Study	Safety factor
ADI	0–0.06 mg/kg bw	Dog, 1-year study of toxicity	100
ARfD	0.1 mg/kg bw	Rabbit, developmental toxicity, (maternal toxicity)	100

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DIAZINON (addendum)

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Explanation

Diazinon is the International Organization of Standardization (ISO) approved name for the contact organothiophosphate insecticide, *O,O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate (International Union of Pure and Applied Chemistry, IUPAC). Diazoxon, the biologically active metabolite of diazinon, inhibits the activity of cholinesterase.

Diazinon has been reviewed by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) on several occasions since the first evaluation in 1963. In 1966, an acceptable daily intake (ADI) of 0–0.002 mg/kg bw per day was established based on a no-observed-effect level (NOEL) of 0.02 mg/kg bw per day for inhibition of plasma cholinesterase activity in a 37–43-day study in humans. In 2001, the Meeting established an acute reference dose (ARfD) for diazinon. Although a new study of acute toxicity in humans was submitted, the ARfD of 0.03 mg/kg bw was based on a no-observed-adverse-effect level (NOAEL) of 2.5 mg/kg bw observed in a study of acute neurotoxicity in rats.

The present Meeting re-considered the ADI and ARfD for diazinon because the existing ADI was based on a study in men only, while a second study in male volunteers was not considered suitable as the basis for an ARfD in 2001. As inhibition of cholinesterase activity is the most sensitive toxicological end-point for diazinon, all previously-considered studies that reported cholinesterase activity and five additional studies were reviewed by the present Meeting. The new data included the final report of the preliminary study of acute toxicity in humans that was considered by the JMPR in 2001, another repeat-dose study in humans, a short-term study of toxicity in rats and two published studies.

Unpublished studies in laboratory animals complied with good laboratory practice (GLP) and with the relevant Organisation for Economic Co-operation and Development (OECD) test guidelines. Studies in humans were conducted in accordance with principles such as those expressed in the

Declaration of Helsinki (Cristie, 2000) or equivalent statements prepared for use by national and/or multinational authorities.

1. Toxicological studies

1.1 Acute toxicity

(a) Results of studies of acute toxicity

The results of studies of acute toxicity are summarized in Table 1. Clinical signs of acute toxicity are consistent with those caused by organophosphates, including decrease of spontaneous activity, sedation, dyspnoea, ataxia, tremors, convulsions, lacrimation and diarrhoea. The symptoms were reversible in surviving animals.

Table 1. Results of studies of acute toxicity with diazinon (technical material)

Species	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/m^3)	Purity (%)	Reference
Mouse	Males and females	Oral	187	—	NS	Bathe (1972a)
Rat	Males and females	Oral	422	—	97.1	Bathe (1980)
			1350 (males) 1160 (females)	—	87.9	Kuhn (1989)
			300	—	95.7	Piccirillo (1978)
			1031 (males) 870 (females)	—	96.1	Schoch (1985)
			> 2150 (no deaths)	—	NS	Bathe (1972b)
		Inhalation	—	> 2327 (no deaths)	87.9	Holbert (1989)

LC_{50}: median lethal concentration; LD_{50}, median lethal dose; NS, not stated.

(b) Time-course of acute inhibition of cholinesterase activity

Rats

Groups of 15 male and 15 female Hsd:Sprague-Dawley rats were given single doses of diazinon (purity, 88%) at a dose of 0, 2.5, 150, 300 or 600 mg/kg bw by gavage. Of the 15 rats, 10 were used for neurological testing and 5 for measurement of cholinesterase activity. Dose selection was based on the results of three studies of median lethal doses that revealed the lowest lethal dose to be 750 mg/kg bw and the highest non-lethal dose to be 500 mg/kg bw. Hence, at the highest selected dose of 600 mg/kg bw, significant neurotoxic effects were anticipated. The selected intermediate doses (150 and 300 mg/kg bw) were reducing multiples of the highest dose and so were anticipated to give intermediate or minimal effects, while a previous investigation (Glaza, 1993) had indicated that no reduction in cholinesterase activity in erythrocytes and brain was evident at the lowest dose of 2.5 mg/kg bw. Groups of 10 male and 10 female rats were given triadimefon (purity, 99%) at a dose of 150 mg/kg bw by gavage as a positive control for neurological effects. On the day of treatment, all rats were observed before and after dosing and twice daily thereafter for general

appearance, behaviour, signs of toxicity, morbidity and mortality. Each week, all rats were examined in detail, including palpation for tissue masses. Body weights were estimated before dosing and weekly thereafter, and food consumption was estimated weekly. A functional observational battery (FOB) test was performed 1 week before administration of the test materials, at the time of peak effect after administration of the test materials (9–11 h for diazinon, 1 h for triadimefon) and 7 and 14 days after administration (of diazinon) on the 10 rats intended for neurological testing. Blood samples were obtained at the estimated time of peak effects and at 14 days for the five rats designated for cholinesterase testing in each group, and plasma and erythrocyte cholinesterase activity was determined (by colorimetric assay), while brain cholinesterase activity was estimated in whole brain samples at termination 14 days after administration. The rats used for neurological examination (survivors to termination and decedents) were subjected to necropsy. Sections were made at 10 levels of the brain, cervical, thoracic, lumbar and sacral spinal cord with ganglia and right and left sciatic nerves, right and left fibular nerves, right and left tibial nerves and right and left lateral cutaneous sural nerves, and the Gasserian ganglion. Sections were also taken of skeletal muscle from the right thigh, eyes with the optic nerve and any gross lesions identified. Only five rats per group in the groups receiving diazinon at 600 mg/kg bw, the control group and those treated with triadimefon were processed for histopathological examination.

Two males and one female died during the study. These deaths were attributed to treatment with diazinon, and all occurred at the highest dose. There was a single accidental death in the group of five rats at 300 mg/kg bw and intended for cholinesterase measurement during blood sampling. One control animal was removed from the study as it was thought to have been wrongly dosed, since it had signs of cholinergic poisoning and low cholinesterase activity. The clinical observations included chromodacryorrhoea, reduced activity and tremors at a dose of 300 mg/kg bw, while the group at 600 mg/kg bw also had chromorhinorrhoea, diarrhoea and pallor. Significant decreases in body-weight gain were observed in males at doses equal to or greater than 300 mg/kg bw, while no effects were observed on body weight or weight gain in the females. Food consumption was decreased in males at doses equal to or greater than 300 mg/kg bw and in females at doses equal to or greater than 150 mg/kg bw. In both males and females, effects on parameters of the FOB test were seen only at the estimated time of peak effect after administration of the test materials (9–11 h for diazinon, 1 h for triadimefon) and not on day 7 or 14 after exposure.

The autonomic parameters affected by diazinon at the time of peak effects in males were faecal consistency and soiled fur at doses equal to or greater than 150 mg/kg bw, and these effects were dose-related. Increased salivation, staining of the nose and repeated opening and closing of the mouth were observed at doses equal to or greater than 300 mg/kg bw. Additionally, impaired respiration, lachrymation and staining of the mouth were seen at 600 mg/kg bw. In females, repeated opening and closure of the mouth were observed at doses equal to or greater than 150 mg/kg bw and altered faecal consistency, soiled fur and staining of the nose were observed at doses equal to or greater than 300 mg/kg bw. At the highest dose, impaired respiration and lachrymation were observed. The neuromuscular parameters that were affected in males were: abnormal gait at equal to or greater than 150 mg/kg bw; ataxic gait, impaired righting reflex, impaired hindlimb extensor reflex and decreased hindlimb footsplay at doses equal to or greater than 300 mg/kg bw and reduced forelimb grip strength at 600 mg/kg bw. In the females, ataxic and abnormal gait was observed at equal to or greater than 150 mg/kg bw and impaired righting reflex at equal to or greater than 300 mg/kg bw. Impaired hindlimb extensor reflex, abnormal hindlimb positioning when held by the tail and reduced forelimb and hindlimb grip strength were observed at 600 mg/kg bw. Central nervous system excitability parameters were also affected. In males, tremors were observed in the home cage and open field at equal to or greater than 300 mg/kg bw, as was twitching or muscle fasciculation. At 600 mg/kg bw, the arousal level was decreased. Females had tremors in the home cage at the highest dose only and in the open field at doses equal to or greater than 300 mg/kg bw. Twitching in the open field was seen at the highest dose and a

lowered arousal level at doses equal to or greater than 300 mg/kg bw. Touch response was reduced in females at the highest dose, and the tail-pinch response was reduced in rats of each sex at this dose. Reduced body temperature was observed in males at doses equal to or greater than 300 mg/kg bw and in females at equal to or greater than 150 mg/kg bw. Additionally, females at equal to or greater than 300 mg/kg bw were dehydrated. Locomotor activity in the figure-of-eight maze decreased over time in all groups. At the estimated time of peak effect, the activity of males at doses equal to or greater than 300 mg/kg bw and of females at doses equal to or greater than 150 mg/kg bw was decreased. At 7 and 14 days after dosing, the mean total activity counts were similar for all groups.

At the time of peak effect after intake of diazinon (9–11 h), plasma cholinesterase activity was diminished in all treated groups; however, no differences were observed between groups 14 days after dosing. Erythrocyte cholinesterase activity was inhibited at doses equal to or greater than 150 mg/kg bw in both males and females at the time of peak effect. In males, the activity was 18%, 17% and 15% of that of concurrent controls at 150, 300 and 600 mg/kg bw, respectively, while in the females the activity was 24%, 23% and 24% that of concurrent controls at the three doses. Partial recovery was seen 14 days after dosing: in males, the activity at 150 mg/kg bw was 91% that of concurrent controls, while it was 66% and 53% that of concurrent controls at 300 and 600 mg/kg bw, respectively; in females, the activity was 89%, 57%, 74% and 65% that of concurrent controls at 2.5, 150, 300 and 600 mg/kg bw, respectively. No significant differences in brain cholinesterase activity were seen among the groups of males. Significantly reduced brain cholinesterase activity at termination was seen in females that had received diazinon at 150 mg/kg bw, in which the activity was 92% that of concurrent controls; however, as the activity in the groups given the higher doses was comparable to that in the concurrent controls, this finding is unlikely to be of biological significance. No gross or microscopic treatment-related abnormalities were seen at necropsy. Triadimefon decreased weight gain and food consumption during week 1 of the study. At the time of peak effect after exposure (1 h), changes in central nervous system excitability parameters (increased incidence of rearing; increased arousal level) were the only changes seen in the FOB test. The NOAEL was 2.5 mg/kg bw on the basis of depressed erythrocyte cholinesterase and behavioural changes at 150 mg/kg bw (Chow & Richter, 1994; Annex 1, reference *94,* amended with reference to original data).

Groups of albino Crl:CD BR/VAF/Plus rats were given diazinon (purity, 87.9%) by gavage. In phase 1 of the study, five males and five females were given diazinon at a dose of 100, 250 or 500 mg/kg bw. Subsequently, in order to identify an NOAEL, groups of five females receiving diazinon at 25 or 50 mg/kg bw were added. In phase 2 of the study, groups of five males received diazinon at a dose of 0, 0.05, 0.5, 1, 10, 100 or 500 mg/kg bw by gavage, and females received a dose of 0, 0.05, 0.12, 0.25, 2.5, 25 or 250 mg/kg bw. In phase 1, clinical observations were carried out 1, 2, 4 and 8 h after administration of the test material and daily thereafter. Body weights were determined before treatment and on days 7 and 14 and also on those that died after 1 day. Cholinesterase activity was not measured in these rats. In phase 2, clinical observations were made 1, 2 and 4 h after adminstration of diazinon, while body weights were measured before treatment and on day 1. The surviving rats from phase 1 were killed on day 14 and subjected to gross necropsy; abnormal tissues were retained for possible histopathological examination. Plasma and erythrocyte cholinesterase activity was measured by colorimetric assay 24 h after treatment in the rats in phase 2, and the rats were then killed and subjected to gross necropsy, abnormal tissues being retained for possible histopathological examination; additionally, the right half of the brain was removed for determination of cholinesterase activity.

One female that received diazinon at the highest dose died during phase 1 of the study. There was no treatment-related effect on body weight. Clinical signs were seen in males at 250 and 500 mg/kg bw and in females at doses of 50 mg/kg bw and greater; signs included miosis, hypoactivity, absence of the pain reflex, red-stained face, yellow-stained urogenital region and soft stools. No pathological changes were observed that could be attributed to treatment. In phase 2, no

deaths occurred. Body-weight loss during 24 h after dosing was greater in males at 500 mg/kg bw and in females at 250 mg/kg bw than in concurrent controls. Clinical signs of toxicity were seen in males at 500 mg/kg bw and in females at 250 mg/kg bw; signs included miosis, hypoactivity, absent pain reflex, staggering gait, excessive salivation, red-stained face and yellow-stained and/or wet urogenital region. The only gross pathological findings of note were yellow staining of the perineum and red paranasal discharge in males at 500 mg/kg bw and in females at 250 mg/kg bw.

Plasma cholinesterase activity was reduced in males at doses of 10 mg/kg bw and greater and in females at doses of 2.5 mg/kg bw and greater. A statistically and biologically significant reduction in erythrocyte cholinesterase activity was seen in males at 100 mg/kg bw (51% of concurrent control value) and 500 mg/kg bw (64% of concurrent control value). In females, erythrocyte cholinesterase activity was depressed at 25 mg/kg bw (65% of concurrent control value) and 250 mg/kg bw (55% of concurrent control value). Brain cholinesterase activity was reduced in males at 500 mg/kg bw (31% of concurrent control value) and in females at 250 mg/kg bw (30% of concurrent control). In females at 25 mg/kg bw, brain cholinesterase activity was 64% that of controls; while this difference was not statistically significant, it may be biologically significant. At necropsy in phase 2, staining of the perineum and red paranasal discharge were seen in rats of each sex at the highest dose. No treatment-related histological lesions were seen. The NOAEL was 2.5 mg/kg bw on the basis of inhibition of brain and erythrocyte cholinesterase activity in females at the next highest dose (Glaza, 1993; Annex 1, reference *94*).

Diazinon (purity, 88%) was administered as a single dose by gavage to Harlan Sprague-Dawley rats in order to determine the time course of inhibition of serum, erythrocyte and brain cholinesterase activity. Groups of 15 males and 15 females were given diazinon at a dose of 0, 2.5, 150, 300 or 600 mg/kg bw. Dose selection was based on the results of a range-finding study (Glaza, 1993) in which in males at 500 mg/kg bw and in females at 250 mg/kg bw, whole brain cholinesterase activity was inhibited by about 70%, 24 h after dosing. Clinical observations were made immediately before blood sampling from the orbital plexus for determination of serum and erythrocyte cholinesterase activity. Five rats of each sex were killed at 3, 5 and 9 h and the remainder at 24 h to obtain brains and spinal cord for determination of cholinesterase activity, the samples being stored at –70 to –90 °C. Acetylcholinesterase activity was measured in the cerebellum, cerebral cortex, striatum and hippocampus and in the thoracic spinal cord by a modification of the method of Ellman et al. (1961).

Survival was unaffected by treatment. At the highest dose, clinical signs were seen at 3 h, with maximum effect at 9 h in males and some recovery after 24 h, and maximum effect after 24 h in females. No significant differences were seen in body weight. The mean percentage reductions in cholinesterase activities are shown in Table 2.

Table 2. Inhibition of cholinesterase activity in rats given diazinon by gavage

Sample	Interval (h)	Mean percentage reduction in cholinesterase activity[a]							
		Male				Female			
		Dose (mg/kg bw)							
		2.5	150	300	600	2.5	150	300	600
Plasma	3	21**	66**	71**	72**	57**	74**	77**	79**
	9	30**	79**	80**	77**	60**	82**	85**	73**
	24	17**	76**	84**	88**	42**	89**	89**	91**
Erythrocyte	3	0	66**	82**	74**	[1]	42**	50**	73**
	9	[1]	76**	78**	81**	40**	68**	78**	74**
	24	11	68**	77**	76**	11	70**	68**	71**

Cerebellum	3	1	51**	76**	80**	7	54**	48**	66**
	9	6	59**	78**	84**	[2]	65**	79**	77**
	24	[3]	45**	60**	80**	0	68**	74**	81**
Cerebral cortex	3	[16]	31	67**	75**	[4]	34*	35**	56**
	9	20*	62**	82**	85**	5	63**	75**	78**
	24	[23]	45**	60**	80**	1	73**	77**	85**
Striatum	3	0	28*	69**	75**	[13]	26*	31**	50**
	9	[10]	65**	77**	85**	[9]	66**	81**	83**
	24	[12]	43**	58**	85**	5	68**	84**	87**
Hippocampus	3	[5]	40**	70**	80**	10	46**	47**	56**
	9	5	57**	76**	84**	[5]	68**	81**	83**
	24	[25]	45**	62**	85**	1	65**	74**	81**
Thoracic spinal cord	3	[10]	27	65**	77**	[8]	39**	33*	49**
	9	[8]	51**	76**	85**	[4]	63**	73**	78**
	24	[9]	42**	50**	81**	[3]	51**	46**	81**

Adapted from Potrepka (1994)

[a] Values in square brackets indicate the extent (%) to which the measured activity was greater than controls.

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's t test).

Plasma cholinesterase activity was decreased by more than 20% at the lowest dose at 3 h and 9 h, with maximum reduction at 9 h in males and females. At 24 h, the cholinesterase activity in rats at the lowest dose was decreased by 17% in males and 42% in females in comparison with that of concurrent controls. Although erythrocyte cholinesterase activity was significantly decreased in females 9 h after dosing at 2.5 mg/kg bw, the absence of any inhibition at 3 h and 24 h and at any time-point in males suggests that this result in females may be an anomalous finding. In an analysis of cholinesterase activity in regions of the central nervous system in rats at the lowest dose, cholinesterase activity in males was never less than 80% that of controls but in one instance (cerebral cortex at 9 h) it was equal to 80% that of controls. In females at the lowest dose, no substantial decrease in central nervous system cholinesterase activity was observed. At the higher doses, significantly decreased activity was observed in all regions and at all times but was usually greater at 9 h and 24 h than at 3 h. The NOAEL for inhibition of brain and erythrocyte cholinesterase activity was 2.5 mg/kg bw (Potrepka, 1994; Annex 1, reference *94* amended with reference to original data).

1.2 Short-term studies of toxicity

Rats

Groups of 50 male and 50 female Wistar rats (aged 6 weeks) were given semi-purified diets containing diazinon (purity, 99.2%) at a concentration of 0 or 2 ppm (equivalent to 0.2 mg/kg bw per day) for 7 days, or, 0 or 25 ppm (equivalent to 2.5 mg/kg bw per day) for 30 days. The diet was prepared before the start of the studies by mixing diazinon suspended in corn oil with a semi-synthetic diet. Food consumption and body weight were recorded twice weekly and clinical signs were monitored daily. Blood for measurements of plasma and erythrocyte cholinesterase activity were collected from random groups of 10 rats at various times (at 3–5 days intervals) except for those at 0 or 25 ppm in the 30-day study, where the same rats were sampled at each bleed. At days 15 and 30 in the 30-day study, brain cholinesterase activity was measured in 6 sacrificed rats per group. All

cholinesterase activities were measured using a radiometric method, with tritiated acetylcholine as the substrate.

There were no clinical signs observed at any dose. Treated rats at 2 ppm or 25 ppm had a similar food consumption and body-weight gain except for female rats at 25 ppm for which mean food consumption increased by 9% from day 15 onwards. The maximum mean percentage reductions in cholinesterase activity measured in the two studies are shown in Table 3.

Table 3. Maximum inhibition of cholinesterase activity in rats given diets containing diazinon

Dietary concentration (ppm)	Duration (days)	Mean percentage reduction in cholinesterase activity[a]					
		Plasma		Erythrocyte		Brain	
		Males	Females	Males	Females	Males	Females
2	7	5	29*	[12]	3	ND	ND
25	30	52*	76*	44*	85*	[3]	6

Adapted from Davies & Holub (1980a)
ND, not determined.
[a] Values in square brackets indicate the extent (%) to which the measured activity was greater than that in controls.
* $p \leq 0.05$

Relative to males, cholinesterase activity in females appeared to be more sensitive to inhibition after exposure to diet containing diazinon. The NOAEL for this study was 2 ppm (equivalent to 0.2 mg/kg bw per day) based on a statistically significant (> 20%) inhibition of erythrocyte acetylcholinesterase activity at the next highest dose of 25 ppm (equivalent to 2.5 mg/kg bw per day) (Davies & Holub, 1980a).

Groups of 50 males and 50 female Wistar rats (aged 6 weeks; mean body weight, 139 g) were given semi-purified diets containing diazinon (purity, 99.2%) at a concentration of 0, 5, 10, or 15 ppm for 92 days. Female rats were selected for study because a previous short-term study had shown them to be more sensitive than males to inhibition of cholinesterase (Davies & Holub, 1980a). In order to increase the accuracy for determining a NOAEL based on inhibition of cholinesterase activity in plasma, erythrocytes and brain, a second and a third study, each with a reduced duration and concentration of diazinon, were performed. The second study involved groups of 16 rats (mean body weight, 149 g) being fed diets containing diazinon at a concentration of 0, 1, 2, 3, or 4 ppm for 42 days. In the third study, groups of 10 rats (mean body weight, 143 g) were fed diets containing diazinon at a concentration of 0, 0.1, 0.5, 1, or 2 ppm for 35 days. Food consumption and body weight were recorded twice weekly and clinical signs were monitored daily.

In the three studies, blood for determination of plasma and erythrocyte cholinesterase activity were collected from groups of 8–10 rats at various time-points (at intervals of approximately 2–5 days). Determination of brain cholinesterase activity was confined to the two studies of longer duration in which satellite groups of six rats were sacrificed at various time-points (intervals of 5–14 days). Cholinesterase activities were measured using a radiometric method with tritiated acetylcholine as the substrate.

There were no clinical signs observed at any dose in any of the three studies. Treated rats in all studies had similar food consumption and body-weight gain except for rats at 0.5 ppm (third study) where mean body-weight gain and food consumption were 12% and 10% respectively less than values for controls. The maximum mean percentage reductions in cholinesterase activity measured in the three studies are shown in the Table 4.

Table 4. Maximum inhibition of cholinesterase activity in rats given diets containing diazinon

Dietary concentration (ppm)	No. of rats per group	Duration (days)	Mean percentage reduction in cholinesterase activity		
			Plasma	Erythrocyte	Brain
0.1	10	35	4	0	—
0.5		35	16*	0	—
1		35	28*	16	—
2		35	42*	12	—
1	16	42	33*	8	NS
2		42	51*	9	NS
3		42	65*	8	NS
4		42	61*	4	NS
5	50	92	75*	18*	2
10		92	80*	38*	6
15		92	85*	55*	2

Adapted from Davies & Holub (1980b)
NS, approximately equivalent to controls.
The apparent significant reduction in cholinesterase activity, which was due an unexplained increase in cholinesterase activity in controls on day 42, was discounted.
* $p \leq 0.05$.

The NOAEL for cholinesterase inhibition in the first study was 5 ppm (equivalent to 0.5 mg/kg bw per day) based on a statistically significant (> 20%) inhibition of erythrocyte acetylcholinesterase activity at the next higher dose of 10 ppm (equivalent to 1 mg/kg bw per day) after dosing for 92 days. The NOAEL for females in the second and third studies can be established at the highest tested doses of 4 ppm (equivalent to 0.4 mg/kg bw per day) and 2 ppm (equivalent to 0.2 mg/kg bw per day) after dosing for 42 and 35 days respectively (Davies & Holub 1980b).

To monitor cholinesterase inhibition in the blood (i.e. plasma and erythrocytes) and regional areas of the brain (cerebellum, cerebral cortex, striatum and hippocampus) of rats after ingestion of diazinon, groups of 15 males and 15 female Sprague-Dawley rats (Crl:CD®BR) were given diets containing technical diazinon (purity, 88%) at a concentration of 0, 0.3, 30, 300, or 3000 ppm for 28 days. Rats were observed twice daily (morning and afternoon) for general health and behavioural changes and a general physical examination was performed weekly. Food consumption and body weight were recorded weekly during exposure. Cholinesterase activity in plasma, erythrocytes, spinal cord (thoracic region) and regional areas of the brain (cerebellum, cerebral cortex, striatum and hippocampus) were measured using a colorimetric assay on days 8, 15 and 29. No deaths occurred and the predominant treatment-related clinical sign first observed on day 8 was muscle fasciculations in both sexes (3 out of 15 males and 14 out of 15 females) at 3000 ppm. Diarrhoea was also observed in 3 out of 15 females from day 8. Body-weight gain in males was significantly reduced ($p \leq 0.01$) by 54%, 29%, 26% and 26% respectively after each week of treatment at 3000 ppm. Females at the same dose also had a reduced weekly body-weight gain of 103%, 40%, 45% and 40% with all except the gain during week 3 being statistically significant ($p \leq 0.01$). Food consumption at 3000 ppm tended to be lower for females, although this reduction was not significantly reduced ($p \leq 0.01$) except during week 1 for males (21%) and females (27%). The average amount of diazinon consumed, calculated from food consumption and average body weight was 0.02, 2.3, 23, and 213 mg/kg bw per day for males at 0.3, 30, 300, 3000 ppm respectively. For females given diets containing diazinon at the corresponding concentrations, the actual doses of diazinon received were 0.02, 2.4, 23 and 210 mg/kg bw per day. The mean percentage reductions in cholinesterase activities for each treatment group and averaged over the three measurements are shown in Table 5.

Significant and dose-related inhibition of plasma and erythrocyte cholinesterase was evident in males and females at concentrations equal to and greater than 30 ppm (2.4 mg/kg bw per day) from week 1 onwards. Cholinesterase inhibition in the brain showed little regional variation although females appeared to be more sensitive with significant dose-related inhibition ($p \leq 0.01$) being observed in all tested brain regions from week 1 at 300 ppm (23 mg/kg bw per day), while significance at the same dose was only apparent in the cerebellum of males. Therefore, in both sexes, cholinesterase inhibition in plasma and erythrocytes was at least an order of magnitude more sensitive to treatment with diazinon than that observed in regional areas of the brain. The NOAEL was 0.3 ppm (equal to 0.02 mg/kg bw per day) on the basis of inhibition of erythrocyte cholinesterase activity at 30 ppm and above (Chang, 1994).

Table 5. Inhibition of cholinesterase activity in rats given diets containing diazinon

Sample	Exposure (weeks)	Mean percentage reduction in cholinesterase activity[a]							
		Male				Female			
		Actual dose (mg/kg bw per day)							
		0.02	2.3	23	213	0.02	2.4	23	210
Plasma	1	14*	59**	88**	96**	10	81**	95**	98**
	2	17	59**	84**	91**	2	81**	93**	97**
	4	5	51**	77**	87**	32	81**	94**	96**
Erythrocytes	1	1	39**	89**	94**	9	38**	86**	94**
	2	0	55**	83**	85**	8	59**	79**	89**
	4	4	58**	64**	74**	[5]	57**	88**	82**
Cerebellum	1	[4]	2	8*	67**	[12]	[14]	49**	88**
	2	6	1	22**	72**	[6]	6	60**	81**
	4	[2]	[6]	15*	70**	1	6	60**	94**
Cerebral cortex	1	15	7	14	77**	[5]	1	47**	92**
	2	2	[16]	0	80**	[6]	[13]	62**	91**
	4	[4]	[3]	10	84**	[7]	6	72**	91**
Striatum	1	1	[4]	13	82**	6	0	58**	95**
	2	11	2	15	82**	4	[3]	73**	95**
	4	[3]	[5]	5	84**	4	9	78**	97**
Hippocampus	1	[5]	[6]	[2]	79**	[3]	[1]	51**	92**
	2	[5]	0	14	79**	5	2	72**	94**
	4	5	9	9	84**	[11]	[2]	71**	94**
Thoracic spinal cord	1	[15]	[10]	12	73**	10	8	44**	89**
	2	[1]	1	8	72**	[4]	1	76**	96**
	4	[3]	3	11	71**	[13]	[21]	62**	89**

Adapted from Chang (1994)

[a] Values in square brackets indicate the extent (%) to which the measured activity was greater than that of controls.

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's t test).

Groups of 15 male and 15 female Sprague-Dawley Crl:CD®(SD)BR rats were given diets containing diazinon (purity, 87.7%) at a concentration of 0, 0.5, 5, 250, or 2500 ppm for 13 weeks. Food consumption and body weight were measured weekly for 2 weeks before and during treatment, and clinical monitoring was performed daily. Water consumption and urine volume were measured during the week before treatment and then again during week 12. Similarly, ophthalmoscopy and an

auditory/physical examination were also performed before treatment and then again during weeks 12 and 14 respectively. Haematology (erythrocyte volume fraction, haemoglobin, erythrocyte count, leukocyte count, leukocyte differential count, thrombocyte count—Heinz body and reticulocyte counts were performed in the control group and groups at 2500 ppm only—platelet count and prothrombin time) and clinical chemistry including cholinesterase activity (by colorimetric assay) were determined during week 13. Clinical chemistry measured blood glucose concentration, blood urea nitrogen, total serum protein, bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, sodium, potassium, chloride, calcium, phosphorus, cholesterol, creatinine, albumin, gamma globulins and the albumin : globulin ratio. Urine analysis included an assessment of the specific gravity, pH, glucose, ketones, protein, bilirubin and an examination of the sediment.

There were no treatment-related deaths. Clinical signs that appeared to be treatment-related occurred only at 2500 ppm, with soft faeces and a degree of hypersensitivity to touch and sound being intermittently observed throughout treatment in both sexes (10 out of 15 males and 15 out of 15 females; 12 out of 15 males and 15 out of 15 females affected, respectively); aggressive behaviour was also noted in 3 out of 15 males. Body-weight loss resulting from treatment was observed at 2500 ppm; reduced body-weight gain was most evident (statistically significant, $p < 0.01$) from day 14 to day 42 in males and day 7 to day 49 in females, so that at the end of treatment males and females were 6% and 13% respectively lighter than concurrent controls. Although no significant changes in water consumption occurred, food consumption was reduced for rats at 2500 ppm, but only during the first week of treatment in males (17%; $p < 0.01$) and for the first 2 weeks in females (31% and 13% respectively; $p < 0.01$). Calculations involving the concentration of diazinon in food, the weight of food consumed and the average group body weight midstudy, enabled the dose in each treatment group to be calculated. For males this was 0.03, 0.3, 15 and 168 mg/kg bw per day respectively, while for females it was slightly higher at 0.04, 0.4, 19 and 212 mg/kg bw per day.

Ophthalmoscopic examination did not reveal any changes attributable to treatment. The haematological assessment in females revealed dose-dependent changes in erythrocytic parameters (i.e. erythrocte count, 1.3%, 1.8%, 2.8% and 9.5%, respectively; haemoglobin concentration, 1.5%, 2%, 3.6% and 4.1% respectively; erythrocyte volume fraction, 0.018, 0.018, 0.044 and 0.077 respectively), although only erythrocyte volume fraction at 250 ($p < 0.05$) and 2500 ppm ($p < 0.01$) achieved statistical significance. A corresponding significant ($p < 0.01$) increase in reticulocytes (3.3-fold) was also observed in females at 2500 ppm (changes at lower concentrations were not examined). Increased leukocyte count ($p < 0.05$) in females at 2500 ppm and eosinophil count ($p < 0.05$) in males at 0.5 ppm were probably incidental findings as there did not appear to be any dose–reponse relationship. Clinical chemistry changes were also characterized by a lack of any dose–response relationship so that in males the reduced cholesterol (by 18%; $p < 0.05$), elevated alanine aminotransferase (by 16%; $p < 0.05$) at 2500 ppm, and reduced sodium (by 1%; $p < 0.05$) observed in rats at 5 ppm may not be attributable to treatment. Similarly, in females, reduced alanine aminotransferase (by 46%: $p < 0.01$), sodium concentration (1.2%; $p < 0.05$), chloride concentration (3.4%; $p < 0.05$) and elevated phosphorus concentration (24%; $p < 0.01$) at 2500 ppm, and reduced alanine aminotransferase activity at 250 (by 36%; $p < 0.05$) and 0.5 ppm (by 39%; $p < 0.05$) may likewise be unrelated to treatment. However, there were reduced cholinesterase activities in erythrocytes, plasma and brain that were attributable to treatment. These mean percentage reductions in cholinesterase activities are shown in Table 6.

There was significant inhibition of cholinesterase activity in the erythrocytes of females and the plasma of males and females at 5 ppm, while cholinesterase activity in the brain was significantly inhibited once the concentration of diazinon in the diet reached 250 ppm in females and 2500 ppm in males. Apart from a significantly increased specific gravity of the urine (males, 2.2%; females, 1.6%;

$p < 0.01$ for both) at 2500 ppm that was associated with non-significant reductions in urine volume (males, 20%; females, 17%) and water consumption (males, 17%; females, 12%), urine analysis was similar among treatment groups.

Table 6. Inhibition of cholinesterase activity in rats given diets containing diazinon

Dietary concentration (ppm)	Mean percentage reduction in cholinesterase activity[a]					
	Plasma		Erythrocyte		Brain	
	Males	Females	Males	Females	Males	Females
0.5	[10]	12	[4]	4	[7]	[2]
5	26**	78**	4	17**	[5]	0
250	89**	97**	27**	41**	4	41**
2500	97**	98**	26**	42**	49**	57**

Adapted from Singh et al. (1988)

[a] Values in square brackets indicate the extent (%) to which the measured activity was greater than that of controls.

** $p \leq 0.01$.

Macroscopic inspection of organs at necropsy revealed no gross abnormalities, although the absolute (15%; $p < 0.05$) and relative-to-body-weight (20%; $p < 0.01$) weight of the liver was significantly increased in females at 2500 ppm. Males in the same group also had increased absolute (4%) and relative-to-body-weight (7%) liver weights, although neither change was not significant. Although neither males nor females at 2500 ppm had significantly increased liver weights relative to brain weight, an increase of 6.5% and 12% respectively suggests a physiological adaptation, an assertion consistent with centrilolobular heptocellular hypertrophy observed in 13 out of 15 females at 2500 ppm (and 3 out of 15 at 250 ppm). The only other significant ($p < 0.05$) organ-weight change was observed for the body-weight relative increase in weight of the kidneys (12%) in females at 2500 ppm.

In conclusion, rats fed diets containing diazinon at 2500 ppm lost body weight, were hypersensitive to touch and sound, and excreted soft faeces. In females at 2500 ppm, increased liver weight resulting from hepatocellular hypertrophy was also observed. The NOAEL was 5 ppm (equal to 0.4 mg/kg bw per day) on the basis of inhibition of erythrocyte and brain cholinesterase activity at dietary concentrations of 250 ppm and higher (Singh et al., 1988; Annex 1, reference *70*, modified with reference to the original data).

Groups of 15 male and 15 female Sprague-Dawley Crl:CD®(SD)BR rats were fed diets containing diazinon (purity, 88%) at a concentration of 0, 0.3, 30, 300, or 3000 ppm for 13 weeks (equal to 0.017, 1.7, 17, or 177 mg/kg bw per day in males and 0.019, 1.9, 19, or 196 mg/kg bw per day in females). Food consumption and body weight were measured weekly during treatment, and clinical monitoring was performed twice daily. All rats were palpated weekly (although the justification for this in a 3-month study was not given) and opthalmoscopy was performed before and at the completion of treatment (though not for the satellite group). Five rats in each group, not scheduled for the FOB tests, were used exclusively as the source of blood to assess the extent of cholinesterase inhibition (by colorimetric assay) during weeks 4, 8, and 13. Regional brain cholinesterase activity (i.e. cerebellum, cerebral cortex plus hippocampus, and striatum) in these five rats was then measured at week 13. Neurological tests, namely FOB and figure-of-eight maze motor activity, were performed in the presence of 'white noise' for 10 rats in each group, 1 week before treatment and again in week 4, 8 and 13 of treatment. The FOB tests comprised home-cage observations, manipulative measurements, open-field and reflex responses, neuromuscular tests and physiological functions. The following parameters were examined:

- Home-cage observations (posture, tremors, convulsions, stereotypy, bizarre behaviour, faecal colour and composition, and gait);
- Manipulative measurements; ease of removal from cage, respiration character, position of hindlimbs when held by tail, pupillary size, lacrimation, staining, eye prominence, palpebral closure, piloerection, fur appearance, salivation, vocalization, and ease of handling;
- Open field tests; arousal, circling, convulsions, gait, stereotypy, tremors, number of defaecations, numbers of rears, number of urine pools, bizarre behaviour and head position;
- Neuromuscular tests; fore and hindlimb grip strength, hindlimb foot splay and hindlimb extensor strength;
- Physiological measurements; body tone, rectal temperature and muscle tone.

After each FOB test series, rats in each group were individually tested in a figure-of-eight maze for spontaneous activity (measured by light-beam interruption). After 13 weeks of treatment the FOB-tested rats were anaesthetized, then sacrificed by whole-body perfusion (with glutaraldehyde) and a gross necropsy was performed. The only tissues removed for histopathological examination were brain, spinal cord with ganglia (at each level, i.e. cervical, thoracic, lumbar and sacral), peripheral nerves (left and right sciatic, fibular, tibial, lateral cutaneous sural), Gasserian ganglion, eyes with associated optic nerves, skeletal muscle and any other gross lesions detected. Since a preliminary histopathological assessment of sections from the controls and rats at 3000 ppm revealed no significant differences other than for some lesions in the nerve roots of the sacral spinal cord, this tissue was the only one examined in detail from rats at 0.3 and 300 ppm.

All rats survived treatment, although clinical signs consistent with OP toxicity were observed in males (i.e. hypersensitivity to touch and sound) and females (i.e. muscle fasciculations and tremors) at 3000 ppm. At this concentration body-weight gain was significantly ($p < 0.01$) reduced for the first 6 weeks in males and 12 weeks in females. This reduced weight gain, associated with a reduction in food consumption that achieved significance ($p < 0.01$) during weeks 1 and 2 in males (average, 15%) and weeks 1, 2 and 4 (average, 14%) in females, resulted in a generally reduced body weight throughout treatment, but which only achieved significance during weeks 1 to 6 (average, 11%) in males and weeks 1–9 (average, 11%) and 11 in females. Significant changes in body weight and food consumption that occurred at other concentrations were considered to be unrelated to treatment because they were transient, lasting no more than 1 week, and had no apparent trend. There were no treatment-related opthalmoscopic findings.

Clear treatment-related FOB test findings were observed for males and females at 3000 ppm. For males, there was reduced forelimb and hindlimb grip strength (average for both, 16%) throughout treatment (i.e. weeks 4, 8 and 13) that did not achieve significance. However, these reductions in limb grip strength that averaged 25% among females, achieved significance ($p < 0.01$) for forelimb grip strength at weeks 4, 8 and 13 and at week 4 for hindlimb grip strength ($p < 0.05$). Hindlimb foot splay was also significantly reduced ($p < 0.05$) by 32% at week 4 and by 26% and 23% (both not statistically significant) at weeks 8 and 13 respectively. Rectal temperature among females was significantly reduced ($p < 0.05$) at week 13 and 4 out of 10 had signs of dehydration at week 4. FOB changes observed at other concentrations were probably unrelated to treatment because they did not appear to be dose-related. Similarly, maze activity among all groups appeared to be unchanged by treatment.

As shown in Table 7, cholinesterase activities in plasma and erythrocytes were significantly reduced ($p < 0.01$) as a function of dose so that, as anticipated, no inhibition was observed at the lowest tested concentration of 0.3 ppm. Relative to erythrocytes, inhibition of plasma cholinesterase activity was generally more pronounced at 30, 300 and 3000 ppm in males and females, except for plasma cholinesterase activity in males at 30 and 300 ppm. Cholinesterase activity in all regions of

the brain was significantly ($p < 0.01$) reduced at 300 and 3000 ppm in females and at 3000 ppm in males. The spurious values observed in the striatum of males at 30 ppm and 300 ppm were attributed to an inconsistent dissection technique for this small brain region.

Table 7. Inhibition of cholinesterase activity in rats given diets containing diazinon for 4, 8 and 13 weeks

Dietary concentration (ppm)	Time-point	Mean percentage reduction in cholinesterase activity[a]									
		Plasma		Erythrocyte		Brain					
						Cerebellum[b]		Cortex/ hippocampus[b]		Striatum[b]	
		Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
0.3	Week 4	[5]	[12]	16	19	—	—	—	—	—	—
	Week 8	[2]	[3],	[11]	[7]	—	—	—	—	—	—
	Week 13	[5]	[5]	11*	[9]	0	0	[2]	[10]	4	26
Mean	Weeks 4–13	[4]	[7]	5	1	—	—	—	—	—	—
30	Week 4	37**	79**	60**	60**	—	—	—	—	—	—
	Week 8	39**	83**	37**	53**	—	—	—	—	—	—
	Week 13	45**	86**	75**	59**	10	[2]	[21]	25*	[89]	18
Mean	Weeks 4–13	40	83	57	57	—	—	—	—	—	—
300	Week 4	72**	91**	71**	84**	—	—	—	—	—	—
	Week 8	78**	94**	86**	81**	—	—	—	—	—	—
	Week 13	79**	95**	84**	75**	14	55**	6	75**	[138]	74**
Mean	Weeks 4–13	76	93	80	80	—	—	—	—	—	—
3000	Week 4	81**	94**	72**	85**	—	—	—	—	—	—
	Week 8	85**	96**	80**	88**	—	—	—	—	—	—
	Week 13	85**	97**	86**	79**	64**	89**	77**	92**	62**	96**
Mean	Weeks 4–13	84	96	79	84	—	—	—	—	—	—

Adapted from Pettersen & Morrissey (1994)

[a] Values in square brackets indicate the extent (%) to which the measured activity was greater than that of controls.

[b] Brain cholinesterase activity was measured after sacrifice in week 13.

* $p \leq 0.05$; ** $p \leq 0.01$

No treatment-related findings were observed after gross necropsy and no additional histopathological lesions apart from the mild focal degeneration of the sacral spinal-cord nerve root axons in 2 out of 10 females at 3000 ppm, detected in the preliminary screen.

In conclusion, rats fed diets containing diazinon at a concentration of 0.3, 30, 300, or 3000 ppm for 13 weeks lost body weight and had characteristic clinical signs of organophosphate poisoning at the highest concentration tested. FOB studies revealed reduced fore and hindlimb grip strength throughout treatment among all rats at 3000 ppm with additional findings, i.e. reduced hindlimb foot splay and rectal temperature, among females. The NOAEL was 0.3 ppm (equal to 0.019 mg/kg bw per day but rounded up to 0.02 mg/kg bw per day) on the basis of significant inhibition of erythrocyte acetylcholinesterase activity at 30 ppm in males and females (Pettersen & Morrissey, 1994).

Dogs

Groups of four male and four female beagle dogs were given diets containing diazinon (purity, 87.7%) at a concentration of 0, 0.1, 0.5, 150 or 300 ppm (equal to 0.0034, 0.02, 5.9, or 10.9 mg/kg bw per day) for 90 days. No treatment-related mortalities occurred. The treatment did not adversely affect food consumption, ophthalmoscopy, haematology, urine analysis, organ weights or macroscopic findings. Clinical signs such as emesis or bloody faeces were sporadically observed in males at 300 ppm and in females at 150 ppm. Reduced body-weight gain was observed in females at 150 ppm and at 300 ppm in both sexes. In males, inhibition of serum cholinesterase activity was observed resulting in activities of 70%, 20% and 15% of that measured in control rats at the end of the study at 0.5, 150 and 300 ppm, respectively. Erythrocytes activities corresponding to 75% and 69% of control activity were measured at 150 ppm and 300 ppm, while in brain acetylcholinesterase activity was 69% and 58% of control activity at 150 and 300 ppm, respectively. In females a reduction in acetylcholinesterase activity was observed at 150 and 300 ppm. Serum and erythrocyte cholinesterase activities were about 18% and 70% of control activity, respectively, at both doses, and brain cholinesterase activity was 70% and 55% that of control activity at 150 and 300 ppm, respectively. Other changes in biochemical parameters consisted of a decrease in total protein at 300 ppm in males. The only microscopic alteration that might have been compound-related was atrophy of the pancreatic acini in one male dog at the highest dose. The NOAEL was 0.5 ppm (equal to 0.02 mg/kg bw per day) on the basis of inhibition of erythrocyte and brain cholinesterase activity at dietary concentrations of 150 ppm and above (Barnes et al., 1988; Annex 1, reference *70*, modified with reference to the original data).

In a study of oral toxicity, Groups of four male and four female beagle dogs) were fed diets containing diazinon (purity, 87.7%) at a concentration of 0, 0.1, 0.5, 150 or 300 ppm (equal to 0.0032, 0.015, 4.7, or 7.7 mg/kg bw per day for males and 0.0037, 0.02, 4.5, or 9.1 mg/kg bw per day for females) for 52 weeks. Owing to lack of body-weight gain, the dietary concentration of 300 ppm was reduced to 225 ppm after 14 weeks of treatment. Mortality was not increased by treatment and haematology, urine analysis, gross pathology and histopathology revealed no changes attributable to treatment. Overt clinical signs of dehydration and emaciation became evident in one male at 300/225 ppm. The symptoms remained although the initial dose was reduced. Reductions in body-weight gain were observed at 150 ppm and higher in males and at 300/225 ppm in females. However, no clear-cut dose–response relationship was evident and the differences attained statistical significance relative to the control group only at certain observation times. Food consumption was reduced at dietary concentrations of 150 ppm and higher, again without a clear dose–response relationship, most probably owing to reduced palatability of the feed admixtures. Treatment-related decreases in acetylcholinesterase activity at doses of 0.5 ppm and higher were found: serum cholinesterase activity was reduced at 0.5 ppm and higher in males and at 150 ppm and higher in females, resulting in activities that were about 20% of that in the control group at 150 and 300/225 ppm in both sexes. Erythrocyte cholinesterase activity was also reduced at 150 and 300/225 ppm, activities corresponding to about 70% of the control activity being measured in both sexes. Brain cholinesterase activity was inhibited to 75% of that of controls in females at 150 ppm, and at 300/225 ppm to 65% and 75% of that of controls in females and males, respectively. The NOAEL was 0.5 ppm (equal to 0.02 mg/kg bw per day) on the basis of inhibition of erythrocyte and brain cholinesterase activity at 150 ppm and above (Rudzki et al., 1991; Annex 1, reference *70*, modified with reference to the original data).

1.3 Long-term studies of toxicity

Rats

Groups of 30 male and 30 female Sprague-Dawley (Crl:VAF/Plus CD[SD]Br) rats (40 males and 40 females in the control group and at 250 ppm) were fed diets containing diazinon (purity,

87.7%) at a concentration of 0, 0 (i.e. with epoxidized soybean oil at 26.5 ppm), 0.1, 1.5, 125, or 250 ppm active ingredient (equal to 0.004, 0.06, 5 and 10 mg/kg bw per day for males and 0.005, 0.07, 6 and 12 mg/kg bw per day for females) for 98/99 weeks. Dose selection was based on the results of short-term study/ies (although not explicitly stated, this reference probably relates to the 13-week feeding study by Singh et al., 1988) so that for long-term dosing the NOEL was anticipated to be 0.1 ppm while the second lowest concentration of 1.5 ppm was anticipated to be the NOAEL with only serum cholinesterase activity being affected. Cholinesterase activities in brain, erythrocytes and plasma were all anticipated to be affected at the highest concentration of 250 ppm. Rats were monitored daily for mortality and clinical signs. Body weight and food consumption were recorded before treatment and then weekly for the first 13 weeks and monthly thereafter. Physical and auditory examinations were performed 2 weeks before testing, and then during weeks 12, 26, 39, 52, 57 (for rats in the recovery group only), 65, 79, 92, 97 (males at 0.1 ppm only) and 98. After 52 weeks of treatment, satellite groups of 10 males and 10 females were sacrificed for an interim necropsy and another10 males and females in the control, vehicle-control group (i.e. with epoxidized soybean oil) and group at 250 ppm were fed the untreated diet for a 4-week recovery period. Interim-recovery rats were then sacrificed for necropsy during week 57. All other rats were sacrificed and necropsied during weeks 98–99 of treatment.

The study was terminated after 98–99 weeks because of decreased survivability in the 0.1 ppm male group only, which was unrelated to treatment and associated with age-related changes (e.g. senile nephropathy and/or pituitary adenoma, both considered to be the result of senescence in this strain of rat). This earlier termination was not considered to have affected the quality or integrity of the study because a sufficient number of animals were at risk in the other treatment groups for the development of tumours. The treatment did not cause increased mortality, and did not affect ophthalmological or haematological findings, urine analysis or organ weights. Body-weight gain was increased in males at doses of 0.1 ppm and higher and in some instances in females at 125 ppm and higher compared with the untreated control rats. Because body-weight gain in the vehicle control group also showed an increase compared with the untreated controls, the increases in body weight may reflect increased palatability of the feed admixtures containing the epoxidized soybean oil. In fact the increases in mean body-weight gain generally coincided with increases in mean food consumption in these groups. Decreases in serum cholinesterase activities were observed at concentrations of 1.5 ppm and higher in both sexes, resulting in values of about 50% of the control activity at the end of the study. A dose-dependent reduction in erythrocyte cholinesterase activity was observed at 125 and 250 ppm, resulting in activities of 80% and 75% that of controls in males and females, respectively, at either dose at the end of the study. Brain cholinesterase activity was also inhibited to 76% and 71% of the control value in males and females, respectively, at 125 ppm, and to 58% and 52% of the control value at 250 ppm in males and females, respectively. Similar inhibition was observed after the first year of the study. A slight reduction of less than 9% was still found after the 4-week recovery period in erythrocyte and brain cholinesterase activity at 250 ppm. Gross and microscopic pathology examinations did not give evidence of any compound-related lesions. The NOAEL was 1.5 ppm (equal to 0.07 mg/kg bw per day) on the basis of inhibition of erythrocyte and brain cholinesterase activity at dietary concentrations of 125 ppm and above (Kirchner et al., 1991; Annex 1, reference *70*, modified with reference to original data).

2. Observations in humans

Studies in volunteers

A double-blind, placebo-controlled study was performed in which healthy, informed male volunteers were given single, ascending doses of diazinon (purity, 97.8%) in corn oil in gelatin

capsules. Some volunteers received only corn oil in gelatin capsules. The lowest dose used was 0.03 mg/kg bw, given initially to one volunteer receiving the test material and one receiving the placebo. In the next phase, one volunteer received the placebo, six received diazinon at a dose of 0.03 mg/kg bw and one received a dose of 0.12 mg/kg bw. In the next phase, one volunteer received the placebo, six received diazinon at a dose of 0.12 mg/kg bw and one received a dose of 0.21 mg/kg bw. In the next phase, one volunteer received the placebo, six received diazinon at a dose of 0.21 mg/kg bw and one received a dose of 0.30 mg/kg bw. In the final phase, three volunteers received the placebo and seven received diazinon at a dose of 0.20 mg/kg bw. A complete physical examination, including an electrocardiogram, was carried out before and 2 days and 15 days after dosing. Vital signs (respiratory parameters, oral temperature, blood pressure and pulse) were recorded before administration of the test material and 1, 2, 4, 6, 8, 12, 24 and 48 h and 4, 7 and 14 days afterwards. The volunteers were asked to report all adverse events. Blood was taken for clinical chemistry and haematological examination before dosing and on days 1, 2 and 15 after dosing. Blood was collected 1 and 2 days and immediately before dosing, and plasma and erythrocyte cholinesterase activity was determined by a modification of the method of Ellman et al. (1961). Further samples for determination of cholinesterase activity were taken 1, 2, 4, 6, 8, 12, 24 and 48 h after dosing and on days 5, 8 and 15 after dosing. Urine samples were collected during 24 h before dosing and for 0–6, 6–12, 12–24 and 24–48 h after dosing.

The only self-reported adverse event considered to be related to intake of the test material was back pain in a man at the highest dose. No treatment-related effects on haematological or clinical chemical parameters were observed, except for changes in cholinesterase activity. Plasma cholinesterase activity was inhibited by more than 20% at doses greater than 0.12 mg/kg bw. No significant inhibition of erythrocyte cholinesterase activity was seen at any dose; at 0.21 mg/kg bw, 7% inhibition was observed at 4 h, 4% inhibition at 8 h and 6–7% inhibition at days 5, 8 and 15. At other times, the erythrocyte cholinesterase activity was greater than or equal to that of the group given the placebo. Furthermore, data for the man given the highest dose did not suggest any significant inhibition of erythrocyte cholinesterase activity. The NOAEL was 0.21 mg/kg bw (the highest dose was ignored, as only one man received it) (Meyer, 1999; Annex 1, reference *94*; final version reported as Anderson, 2000).

The results of the following study by Lazanas et al., (1966) were reported in an earlier monograph (Annex 5, reference 7). Because of certain inaccuracies in that monograph, the study was reviewed again and summarized below.

Three healthy adult male volunteers (Nos 4, 5 and 6) were given gelatin capsules containing a mixture of diazinon 50W (50% w/w wettable powder; purity, 49.6%) and corn starch at a final dose of 0.025 mg/kg bw per day administered as three doses per day, taken before meals at 08:00, 12:00 and 18:00, for 43 days. A concurrent control group of three volunteers (Nos 1, 2 and 3) were given gelatin capsules containing corn starch only. In a second dosing regimen, three different volunteers (Nos 1, 7 and 8) were treated as above at a dose of 0.020 mg/kg bw per day for 37 days. In the recovery phase, after treatment at 0.025 mg/kg bw per day, volunteers were given capsules containing corn starch only (placebo) for 101 days; this recovery phase was reduced to 41 days for the group at 0.020 mg/kg bw per day. Plasma and erythrocyte cholinesterase activities in all eight volunteers were measured on five separate occasions before dosing and then at intervals of 1–5 days throughout treatment and recovery using an electrometric method (Δ pH/h). Body weight and clinical signs were monitored daily. Haematological parameters, including haemoglobin concentration, erythrocyte count, total and differential leukocyte count and prothrombin time were determined at intervals of 4–7 days during treatment and at 4–14 days during recovery, as were clinical chemistry parameters, i.e. cholinesterase activity (erythrocytes and plasma), blood urea nitrogen, alkaline phosphatase and alanine aminotransferase, and urine analysis, i.e. pH and microscopic elements.

No clinical signs or changes in body weight were observed. No significant changes were detected in any of the haematological, urinary or clinical chemistry parameters measured, except for plasma cholinesterase activity (average inhibition, approximately 22%). By contrast, mean erythrocyte cholinesterase activity was not appreciably inhibited (maximum, 2%) at any time during treatment at 0.025 mg/kg bw per day or during the 22 days of recovery.

Treatment at 0.020 mg/kg bw per day resulted in a combined non-significant mean plasma cholinesterase inhibition of 8% and recovery appeared to be complete after 16 days. Similarly, erythrocyte cholinesterase activity was not significantly affected by treatment at 0.020 mg/kg bw per day. The NOAEL was 0.025 mg/kg bw per day on the basis of depression of plasma cholinesterase activity, the only effect observed at this dose (Lazanas et al., 1966, referenced in Annex 5, reference 7 as Ind. Biotest. Lab., 1966; amended with reference to original data).

The results of the following study by Payot (1966) were reported by the JMPR in 1966 and 2001. Because of some inaccuracies in reporting the administered doses, an amended summary of the study is given below.

Four adult male volunteers (age 30–45 years; body weight, 66, 74, 91 and 95 kg respectively) were given gelatin capsules containing 0.5 mg of technical diazinon (purity, 95.4–95.7%) post-prandially. Since the amount of diazinon in each capsule was fixed and volunteers were given either four or five capsules depending on whether their body weight was closer to 75 or 100 kg respectively, the actual administered doses varied slightly, i.e. 0.03, 0.027, 0.022/0.027 (alternate day treatment with four and five capsules) and 0.026 mg/kg bw per day, respectively. To ascertain reversibility of effects, after complete plasma cholinesterase inhibition in two of the four volunteers was observed after 1 day of treatment, dosing was suspended on days 5–10 of the 42-day regimen, while the other two (lighter) volunteers who commenced treatment a month later were treated uninterrupted for 34 days.

Plasma cholinesterase activity was markedly depressed the first 6 days of treatment in two out of four subjects (no plasma cholinesterase activity measurable). Treatment was therefore interrupted for 6 days to enable recovery. Subsequent treatment at the same dose did not reveal any inhibitory effects on plasma cholinesterase activity. The fluctuations observed during treatment were similar to those observed during the pre-test period. In no instance was erythrocyte acetylcholinesterase activity depressed compared with the pre-test values. Other parameters investigated included haematology and blood chemistry, urine analyses and symptomatology. No changes were observed that could be attributed to treatment. The NOAEL was 0.03 mg/kg bw per day on the basis of transitory depression of plasma cholinesterase activity, the only effect observed at this dose (Payot, 1966; Annex 5, references *6, 94*, amended with reference to original data).

In a preliminary study, four healthy adult male volunteers (age not specified; body weight, 79.5, 83.5, 84 and 95 kg, respectively) were given gelatin capsules containing either 2.47 mg or 2.85 mg of technical diazinon (purity, 99.5%). The study was conducted in accordance with principles such as those expressed in the Declaration of Helsinki or equivalent statements prepared for use by national and/or multinational authorities (Cristie, 2000). Volunteers were given only one capsule containing either the greater or lesser amount, depending on whether their body weight was closer to 95 or 85 kg respectively. The dose administered was 0.03 mg/kg bw per day for 28, 29 or 31 days, depending on the participant's agenda. The administered dose was selected on the basis of the Payot study (Payot, 1966), but it was slightly increased because the purity of diazinon had increased with a concomitant reduction in its acute toxicity. The exclusion criteria included clinically relevant cardiovascular, renal, haematological, or biochemical profiles (including plasma cholinesterase activity) and metabolic or gastrointestinal disorders likely to influence absorption. Individuals with an abnormal electrocardiogram or who were positive for HIV or hepatitis B were also excluded.

Blood was taken for measurement of haemoglobin concentration, erythrocyte, leukocyte, and platelet counts, blood urea nitrogen, glucose, sodium, potassium, chloride, bicarbonate, cholesterol, creatinine, triglyceride, and bilirubin concentrations, and alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, creatine kinase, and gamma-glutamyl transferase activities before dosing. Blood for estimating cholinesterase activity was collected on four occasions (days −28, −27, −7 and −1) before dosing and then 6 h after dosing. Thereafter activity was measured on days 1, 2, 3, 8, 13/14, 20, 28, 29 or 30. Clinical investigations, haematology, clinical chemistry and urine analysis were performed on days 8, 13/14, 20, 28, 29 or 30.

No cholinergic signs were observed. Similarly, there were no treatment-related clinical changes in haematological or clinical chemical end-points, apart from cholinesterase activity during the study. Plasma cholinesterase activity was reduced by an average of 48% relative to pre-test values in all four volunteers on day 20 of treatment. A significant reduction in plasma cholinesterase activity (> 20%) occurred by day 8 of treatment. There was no reduction in erythrocyte acetylcholinesterase activity at any time during treatment. In view of the absence of cholinergic signs or inhibition of acetylcholinesterase activity in erythrocytes, the NOAEL was 0.03 mg/kg bw per day (Beilstein, 1998).

Comments

Toxicological data

The oral LD_{50} for diazinon in rats ranged from 187 to 1160 mg/kg bw, while the dermal LD_{50} and inhalation LC_{50} were > 2150 mg/kg bw and > 2.3 mg/l, respectively.

Signs of acute toxicity after oral, dermal, or inhalational administration were those typically observed with most organophosphorus cholinesterase-inhibiting pesticides and included muscarinic effects (diarrhoea, salivation, pupil constriction), nicotinic effects (muscle fasciculations and fatigue) and central nervous system effects (ataxia, convulsions).

The most sensitive end-point observed in all species given single and repeated doses of diazinon was inhibition of cholinesterase activity. In studies designed to establish the time course of clinical signs and inhibition of cholinesterase activity in rats, there was an apparent sex difference in sensitivity to clinical signs, with females showing signs of poisoning at doses of 50 mg/kg bw or greater, while in males it was at doses of 250 mg/kg bw or greater. However, there was little difference in inhibition of cholinesterase activity between the sexes. The overall NOAEL in all studies of acute toxicity was 2.5 mg/kg bw on the basis of inhibition of cholinesterase activity in erythrocytes and in the brain at the next highest dose in both sexes.

In some repeat-dose dietary studies, female rats appeared to be more sensitive to inhibition of cholinesterase activity, while lacking clinical signs. This apparent sex difference in sensitivity for cholinesterase inhibition was confirmed in a 28-day dietary exposure study in rats in which cholinesterase activity was monitored in the blood and in regional areas of the brain. Significant dose-related reductions in erythrocyte and plasma cholinesterase activity were observed at 30 ppm (2.3 mg/kg bw per day) in both sexes, while significant dose-related inhibition of cholinesterase activity was observed in all tested regions of the brain (i.e. cerebellum, cerebral cortex, striatum, hippocampus and thoracic spinal cord) of females at 300 ppm (23 mg/kg bw per day), but only in the cerebellum of males. The greater incidence of treatment-related muscle fasciculations among females (14 out of 15) relative to males (3 out of 15) at the highest tested dose of 3000 ppm (210 mg/kg bw per day) is additional support for a sex difference in sensitivity.

In a 1-year study in dogs, clinical signs and reduced body-weight gain were observed in females at slightly lower doses, i.e. 150 ppm (5.9 mg/kg bw per day) in females and 300 ppm (10.9 mg/kg bw per day) in males.

The lowest-observed-adverse-effect level (LOAEL) for cholinesterase inhibition in erythrocytes in repeat-dose studies was 10 ppm (equivalent to 1 mg/kg bw per day) in a 92-day dietary study in rats. The highest NOAEL in the database was 5 ppm (equivalent to 0.5 mg/kg bw per day) in the 92-day study in rats.

In a study of acute toxicity in male volunteers given ascending doses of diazinon, cholinesterase activity was not inhibited in erythrocytes at 0.21 mg/kg bw, the second highest dose tested. The highest dose was ignored, as the group comprised a single volunteer. Repeat-dose studies in male volunteers given diazinon for 28–37 days showed that, while there was some inhibition of plasma cholinesterase activity at the highest tested dose of 0.03 mg/kg bw per day, no inhibition of erythrocyte cholinesterase activity was observed.

Toxicological evaluation

The Meeting identified inhibition of cholinesterase activity as the most sensitive end-point observed after single or repeated doses of diazinon in all species. The LOAEL for reduced cholinesterase activity in erythrocytes was 1 mg/kg bw per day and the NOAEL was 0.5 mg/kg bw per day. In considering the NOAEL and LOAEL identified in all repeat-dose studies, the Meeting concluded that all species appeared to be equally sensitive and the extent of cholinesterase inhibition was not dependent on duration of dosing, once steady-state had been achieved within 4 weeks. After considering all previously evaluated data and the new studies, the Meeting established an ADI of 0–0.005 mg/kg bw based on the highest NOAEL of 0.5 mg/kg bw per day for inhibition of erythrocyte cholinesterase activity in a 92-day repeat-dose study in rats, and with a safety factor of 100.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity[b]	Behavioural changes and cholinesterase inhibition in erythrocytes	2.5 mg/kg bw	150 mg/kg bw
	Time course of cholinesterase inhibition after a single dose[b]	Cholinesterase inhibition in erythrocytes and brain	2.5 mg/kg bw	25 mg/kg bw
Human	Acute toxicity[c]	No inhibition of cholinesterase activity	0.21 mg/kg bw	—
Rat	3-month studies of toxicity[a]	Cholinesterase inhibition in erythrocytes and brain	5 ppm, equal to 0.4 mg/kg bw per day	250 ppm, equal to 19 mg/kg bw per day
		Cholinesterase inhibition in erythrocytes[c]	5 ppm, equivalent to 0.5 mg/kg bw per day	10 ppm, equivalent to 1 mg/kg bw per day
	98–99 week study of toxicity[a]	Cholinesterase inhibition in erythrocytes and brain	1.5 ppm, equal to 0.07 mg/kg bw per day	125 ppm, equal to 6 mg/kg bw per day
Dog	1-year study of toxicity[a]	Cholinesterase inhibition in erythrocytes and brain	0.5 ppm, equal to 0.02 mg/kg bw per day	150 ppm, equal to 4.5 mg/kg bw per day

Human	37 days	No inhibition of cholinesterase in erythrocytes	0.025[c]	—
	34 days		0.03[c]	
	28 days		0.03[c]	

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

The Meeting reaffirmed the ARfD of 0.03 mg/kg bw established by the 2001 JMPR. This ARfD was based on the NOAEL of 2.5 mg/kg bw identified in studies of acute toxicity and neurotoxicity in rats, and a safety factor of 100. This ARfD was supported by the NOAEL of 0.21 mg/kg bw identified in the study in humans given a single dose of diazinon, and a safety factor of 10.

Estimate of acceptable daily intake for humans

0–0.005 mg/kg bw

Estimate of acute reference dose

0.03 mg/kg bw

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

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

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HALOXYFOP (INCLUDING HALOXYFOP-R AND HALOXYFOP-R METHYL ESTER)

First draft prepared by
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Explanation

Haloxyfop is the International Organization of Standardization (ISO) approved name for (*R/S*)-2-[4-(3-chloro-5-trifluoromethyl-2-pyridyloxy)phenoxy]propionic acid. It is a substituted phenoxypropionic acid derivative that was developed as a selective herbicide for control of grass weeds in broadleaf crops. In the first formulations produced, the active substance was either racemic haloxyfop ethoxyethyl ester or the racemic methyl ester. As it has been demonstrated that haloxyfop-R is the herbicidally active isomer, and essentially no activity is associated with the *S* isomer, a resolved methyl ester has been developed which is approximately 98% *R* isomer. When applied to plants, the esters are rapidly hydrolysed to the acid.

Haloxyfop (racemic), its sodium salt and its esters (racemic haloxyfop ethoxyethyl ester and racemic haloxyfop methyl ester) were first evaluated by the JMPR in 1995, when the Meeting established an acceptable daily intake (ADI) of 0–0.0003 mg/kg bw based on a no-observed-adverse-effect level (NOAEL) of 0.03 mg/kg bw per day for liver tumours in a 2-year study in mice. New toxicological data had been made available since this date. Haloxyfop was re-evaluated at the request of the Codex Committee on Pesticide residues (CCPR). New data on pharmacokinetics, dermal toxicity, genotoxicity and special studies of hepatocellular peroxisome proliferation had become available since the last evaluation.

All studies with haloxyfop-R methyl ester and pivotal studies with haloxyfop were certified as being compliant with good laboratory practice (GLP). Other studies were carried out before the Organisation for Economic Co-operation and Development (OECD) guidelines on GLP were promulgated. The quality of these studies was considered to be acceptable.

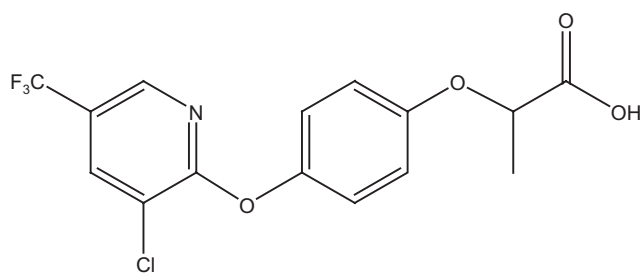
Numerous studies of the pharmacology and toxicology of various chemical forms of haloxyfop were available. Investigations with haloxyfop-R and its methyl ester were limited to studies of absorption, distribution, metabolism and excretion, acute toxicity, short-term studies of toxicity, and genotoxicity.

Haloxyfop-R methyl ester and haloxyfop-R are used as active ingredients in herbicide products used on crops, including carrots, fodder legumes, rapeseed, soya bean and sugar beet. The *R*-isomer is the herbicidally-active form of the chemical. Haloxyfop-R methyl ester and haloxyfop-R are the only forms of haloxyfop that are now manufactured and registered globally, although racemic forms and the ethoxyethyl ester have been used in the past. Commercially produced haloxyfop-R methyl ester contains a minimum of 98% of *R*-isomer and a maximum of 2% *S*-enantiomer.

The CAS Nos for these substances are: haloxyfop methyl ester, 69806-40-2; haloxyfop-R methyl ester, 072619-32-0; haloxyfop acid, 69806-34-4; and haloxyfop ethoxyethyl ester (haloxyfop-etotyl), 87237-48-7. The structural formula of haloxyfop acid is shown in Figure 1.

Chemical names (IUPAC) for these substances are as follows: racemic haloxyfop acid: (*RS*)-2-[4-[3-chloro-5-trifluoromethyl-2-pyriyloxy)phenoxy]propionic acid; haloxyfop-R methyl ester, methyl (*R*)-2-[4-[3-chloro-5-trifluoromethyl-2-pyriyloxy)phenoxy]propionate; racemic haloxyfop ethoxyethyl ester, ethoxyethyl (*RS*)-2-[4-[3-chloro-5-trifluoromethyl-2-pyriyloxy)phenoxy]propionate.

Figure 1. Structural formula of haloxyfop acid



Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

In a study of pharmacokinetics that complied with GLP, groups of 21 male and 21 female B6C3F$_1$ mice were given the sodium salt of racemic haloxyfop as a single oral dose at 5 mg/kg bw. Groups of three mice of each sex were killed at 6, 12, 24, 48, 72, 96 and 168 h after dosing for measurement of the radioactivity in the plasma, kidney and liver. Urine and faeces were collected at 24-h intervals for measurement of radioactivity. Peak plasma concentrations of 23 (males) and 24 mg eq/g (females) were attained at 6 h after dosing. The half-life for apparent first-order absorption into the plasma was 1.5 h for males and 1.9 h for females. Disappearance of radiolabel from the plasma appeared to follow first-order kinetics with half-lives of 1.7 and 1.9 days in males and females respectively and volumes of distribution (V_d) of 196 and 194 ml/kg. The half-lives for removal from the liver and kidneys were respectively 1.7 and 1.8 days in males and 1.9 and 2.0 days in females. At 168 h after dosing, 18% (males) and 24% (females) of the administered radiolabel had been recovered in urine and 66% (males) and 60% (females) had been recovered in faeces. The proportion of the excreted radiolabel that was found in the faeces was 79% for males and 71% for females (Smith et al., 1984).

Rats

In a dose range-finding study, groups of Fischer 344 rats were given racemic haloxyfop methyl ester (radiochemical purity > 98%) that was labelled with ^{14}C on the phenyl ring. Males were given single intravenous or oral doses at either 0.5 mg/kg bw or 50 mg/kg bw, while females were received doses of 10 mg/kg bw orally or intravenously. Urine and faeces were collected at frequent intervals throughout the study. Three rats from each group were killed at 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96 and 120 h after dosing in order to obtain samples of plasma. The oral doses were rapidly absorbed and there was little difference between the pharmacokinetic behaviours of oral and intravenous doses. The clearance of radioactivity from the plasma seemed to follow first-order kinetics. Pharmacokinetics results for appearance and disappearance of radioactivity in plasma are summarized in Table 1. Most of the radioactivity remaining in the bodies of male rats at 5 days after dosing was in the carcass (19.3–25.0% of the administered dose), liver (9.8–18.0%) and skin (10.3–14.6%). The proportion of the excreted radiolabel that was found in the faeces was 63–73% for males and in females the proportion in urine was 68–78% (Smith et al., 1982).

Table 1. Pharmacokinetics in a dose range-finding study in mice given a single dose of radiolabelled racemic haloxyfop methyl ester

Parameter	Males		Females		Males	
	0.5 mg/kg bw, oral	0.5 mg/kg bw, IV	10 mg/kg bw, oral	10 mg/kg bw, IV	50 mg/kg bw, oral	50 mg/kg bw IV
Absorption half-life (h)	1.8	—	1.7	—	3.0	—
Plasma clearance half-life (days)	3.6	3.9	1.1	1.0	2.8	4.5
Volume of distribution (ml/kg bw)	—	236	194	199	—	179
Peak plasma concentration (μg/g)	1.13	2.22	50	50	213	299

Recovery of radioactivity in urine (%)						
3 days after dosing	—	—	45.1	57.0	—	—
5 days after dosing	13.4	11.1	—	—	16.3	15.1
Recovery of radioactivity in faeces (%)						
3 days after dosing	—	—	21.4	15.7	—	—
5 days after dosing	29.0	30.2	—	—	27.7	27.4

From Smith et al. (1982)
IV, intravenous.

In a study that complied with GLP, Fischer 344 rats were given racemic haloxyfop methyl ester labelled with ^{14}C on the phenyl ring (radiochemical purity, > 98%) as a single oral dose at 0.1 mg/kg bw by gavage. Urine and faeces were collected daily throughout the study. Groups of three males were killed for blood sampling at 1, 4, 7, 10, 13, 16, 21 and 24 days after dosing and groups of three females were killed for sampling of liver, kidney, skin, fat, brain, heart, muscle and spleen at 1, 2, 3, 4, 5, 10, 16 and 21 days. One day after dosing, mean concentrations of radioactivity (expressed as equivalents to haloxyfop methyl ester) in plasma were 0.60 µg/g in males and 0.33 µg/g in females. The absorption half-lives were 4.6 h in males and 2.7 h in females. The volumes of distribution (V_d) were 176 ml/kg in males and 251 ml/kg in females. At 1 day after dosing, concentrations of radioactivity were found in the following tissues in decreasing order: liver (0.608 µg eq/g), plasma (0.597 µg eq/g), kidney (0.345 µg eq/g), erythrocytes (0.128 µg eq/g), heart (0.101 µg eq/g), skin (0.081 µg eq/g) and spleen (0.063 µg eq/g), with the relative concentrations remaining in this order throughout the study. The half-lives for clearance of radiolabel from these tissues are shown in Table 2. It was not possible to determine half-lives for skin and spleen in females as the residue concentrations were below the limit of detection after the first day (Smith et al., 1982).

Table 2. Clearance of ^{14}C-radiolabel from tissues of rats given radiolabelled racemic haloxyfop methyl ester as a single dose by gavage

Tissue	Half-life (days)	
	Males	Females
Liver	5.87	1.55
Plasma	5.55	1.59
Kidney	6.13	1.34
Erythrocytes	5.82	1.23
Heart	6.73	—
Skin	7.30	—
Spleen	5.78	—

From Smith et al. (1982)

A series of GLP-compliant experiments was performed to determine the pharmacokinetics of haloxyfop methyl ester (purity, 99.6%) in Fischer 344 rats.

Firstly, two males and two females were given racemic [^{14}C]haloxyfop methyl ester (uniformly labelled on the phenyl ring; radiochemical purity, > 99%) as an oral dose at 2.5 mg/kg bw to determine the time-plasma concentration profiles of radiolabel, haloxyfop methyl ester and haloxyfop at 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing. Urine and faeces were collected at 12 h and 24 h. Haloxyfop methyl ester was not found in any of the samples of blood plasma, while haloxyfop was found at concentrations that matched the amounts of radiolabel found. The Meeting concluded that all of the radiolabel in plasma was in the form of haloxyfop. Peak plasma concentrations of haloxyfop occurred at 8 h after dosing in males and females, with a half-life for absorption of 4.5 h. The haloxyfop was

excreted into urine with an elimination half-life of 23.3 h. The V_d was 185 ml/kg. Urinary excretion occurred more rapidly in females than in males, with 23% of the administered dose being excreted into the urine in the 24 h after dosing by females and only 1.4% in males. Faecal excretion was erratic, with no discernible difference between the sexes.

Secondly, 12 male and 12 female rats were given racemic ^{14}C-haloxyfop methyl ester as a single oral gavage dose at 0.104 mg/kg bw (equimolar to a dose of 0.1 mg/kg bw of haloxyfop) to investigate the routes and rates of excretion, tissue distribution and time-plasma concentration of ^{14}C profiles over the 15 days after dosing. Groups of three males were killed on days 1, 5, 10 and 15 for sampling of plasma, erythrocytes, kidney, liver and adipose tissue for analysis of radioactivity. Similar samples were taken from groups of three females on days 1, 3, 5 and 7. Urine and faeces were collected each day. Plasma concentrations of ^{14}C peaked on day 1 at 0.44 µg eq/g in males and 0.40 µg eq/g in females. Plasma ^{14}C decreased inline with first-order kinetics, but at different rates for males (half-life, 1 day) and females (half-life, 5.5 days). The highest concentration of ^{14}C was found in the plasma, followed by liver, kidney, erythrocytes and adipose tissue in that order, with the amount in adipose tissue being 5–30 times less than in plasma, representing less than 0.2% of the administered material. The plasma clearance half-life was 6.41 days in males and 1.21 days in females and the clearance half-lives for liver and kidney were similar to these values. Urinary and faecal excretion half-lives were 4.8 days and 5.8 days in males and 1.1 days and 1.3 days in females. Faecal excretion was the major route of excretion in males with 59.6% of the administered dose recovered in faeces and 18.6% in urine. In females, the urinary route of excretion was predominant, with 23.5% in faeces and 66.5% in urine. The proportion of the excreted radiolabel that was found in the faeces was 76% for males and for females the proportion found in the urine was 74% (Waechter et al., 1982).

The pharmacokinetics of haloxyfop-R methyl ester in rats given repeated doses was investigated in a GLP-compliant study. Groups of four male and four female Fischer 344 rats were given unlabelled haloxyfop-R methyl ester (purity, 98.6%) as 14 daily oral doses at 0.1 mg/kg bw by gavage, followed by a single oral dose of ^{14}C-labelled haloxyfop-R methyl ester (radiochemical purity, 97.9%) at 0.1 mg/kg bw. During the 7 days after treatment, urine and faeces were collected. At 7 days after dosing, the rats were killed and samples of blood were taken. Autopsies were performed and samples of kidneys, liver, skeletal muscle, skin, and perirenal fat were taken. The amounts of radiolabel in the urine, faeces, plasma, tissues and residual carcass were measured. In addition, urine and faeces from selected intervals, liver, kidney and terminal plasma were analysed for metabolites by high-performance liquid chromatography (HPLC). The results showed that 93% (males) and 89% (females) of the administered radiolabel had been absorbed from the gut. The tissues with the highest concentrations of radiolabel were liver and kidney. The faecal route of elimination predominated in males, while in females the urine was the major route of excretion. In males at 7 days after dosing, 12.4% of the administered dose was excreted in the urine and 41.5% was recovered in the faeces. In females, 74.4% was in the urine and 21.6% in the faeces. Both urinary and faecal excretion of radiolabel occurred more rapidly in females than in males. The half-life for urinary excretion and for faecal excretion was 3.9 days for males; while in females the half-life for urinary excretion was 1.0 days and that for faecal excretion was 1.8 days. The proportion of the excreted radiolabel that was found in the faeces was 77% for males and for females the proportion in the urine was 78% (Mendrala & Hansen, 2001).

The aspects of this study relating to metabolism are summarized in section 1.2.

The dermal absorption of racemic haloxyfop methyl ester by Fischer 344 rats was investigated in a GLP-compliant study. A solution of racemic [^{14}C]haloxyfop methyl ester (purity, 99.0%; uniformly labelled on the phenyl ring) was prepared in acetone and was applied to the clipped but unabraded

skin of nine male and nine female rats. When the acetone had evaporated, the application site was covered with Saran film and an occlusive bandage. Urine and faeces were collected. Groups of three rats were killed for sampling of blood and liver at days 0.5, 2 and 5 for males and days 0.5, 1 and 2 for females. The test material was readily absorbed, with 16.7% and 27.7% of the administered radioactivity being absorbed by males and females over 2 days and 22.5% by males over 5 days. The radiolabel was detected in all samples of blood plasma, but haloxyfop methyl ester was absent from all samples of plasma. It was presumed that the ^{14}C was in the chemical form of [^{14}C]haloxyfop (as seen in the studies using oral administration), but this was not measured. In males, the major route of excretion was the faeces, with 0.5% of the administered dose recovered in the urine and 0.8% in faeces by 2 days after dosing and 1.7% and 6.0% respectively after 5 days. In females, the urine was the major route with 8.3% of the administered dose in the urine and 2.0% in the faeces at 2 days after dosing. The Meeting concluded that haloxyfop methyl ester can be absorbed across the skin and that the kinetics of the absorbed material are similar to the kinetics of orally administered doses. The proportion of the excreted radiolabel that was found in the faeces was 62% for males and for females the proportion in the urine was 81% (Ramsey et al., 1983).

Dogs

In an attempt to explain why dogs appear to be less sensitive to the effects of haloxyfop than do rats and mice, a study of pharmacokinetics was performed in beagle dogs. The study complied with GLP. Two male dogs were given a single oral gavage dose of 2.4 mg/kg bw of racemic haloxyfop acid (purity = 98.8%) that was radiolabelled with ^{14}C uniformly on the phenyl ring (radiochemical purity > 99%). Samples of urine and faeces were collected daily, and blood was collected at 0.5, 1, 2, 4, 6, 8, 12, 31, 48, 72, 144 and 192 h after dosing. The dogs were killed after 8 days and samples of liver, kidney, perirenal fat and bile were taken. The various samples were analysed for radioactivity (^{14}C) and by HPLC and gas chromatography (GC) for metabolites. [^{14}C]Haloxyfop was rapidly and completely absorbed into the blood plasma with a half-life of 9 min. A peak plasma concentration of 23 µg eq/g was reached at 0.5 h after dosing. Average concentrations of radioactivity in samples taken at termination were: bile, 8.49 µg eq/g; liver, 0.51 µg eq/g; plasma, 0.20 µg eq/g; kidney, 0.18 µg eq/g; and perirenal fat, 0.14 µg eq/g. Plasma clearance appear to be biphasic with an initial phase lasting 6–8 h with a half-life of 1–2 h and the second phase having a longer half-life of 34 h. After 8 days, 76.9% of the administered radioactivity had been found in the faeces and 10% in the urine. Of the excreted radiolabel, 88% was in the faeces (Nolan et al., 1987).

The results of the chemical analyses of the samples of organs and excreta that were taken in this study are described in section 1.2.

Monkeys

In a GLP-compliant study, cynomolgus monkeys were given the sodium salt of racemic haloxyfop acid (purity, 99.6%), either unlabelled or labelled with ^{14}C (radiochemical purity, 98.5%), as a single dose at 1 mg/kg bw administered by nasogastric intubation, a second single dose being administered 10 weeks later. The results indicated that the test materials were rapidly absorbed from the gastrointestinal tract with a half-life of less than 30 min. The concentrations of haloxyfop and radioactivity in the plasma and urine were similar at various time-points after dosing. This similarity was taken by the authors to indicate that most of the radioactivity was associated with haloxyfop that had either not been metabolized or conjugates that had become uncoupled during sample preparation. Peak plasma concentrations of 10 µg/g were observed 1–2 h after dosing at 1 mg/kg bw. Haloxyfop was excreted, mainly (84%) in the urine, in a bi-exponential manner; the half-life for the initial phase was 2.5 h and that of the slower second phase was 3 days. Most (95%) urinary excretion occurred in the 24 h after dosing and concentrations were below the limit of quantification (150 ng/g) by 96 h. Only 0.5% of the administered dose of radiolabelled material was

found in the faeces over the 6 days after dosing. Thus more than 99% of the excreted radiolabel was in the urine (Gerbig et al., 1985).

Humans

A GLP-compliant study of pharmacokinetics was performed in three segments.

The first segment was a pilot study in which one male volunteer was given the sodium salt of racemic haloxyfop acid as a single oral dose at 0.2 mg/kg bw. Samples of blood and urine were collected at frequent intervals up to 528 h after dosing. Faeceal samples were also collected, but were not analysed.

The second segment was performed in three male volunteers who were given the sodium salt of racemic haloxyfop as a single oral dose at 0.2 mg/kg bw. Samples of blood and urine were collected at frequent intervals up to 20 days after dosing.

The third segment was performed in four male volunteers who were given racemic haloxyfop methyl ester as a single dermal dose at 0.8 mg/kg bw. Samples of blood and urine were collected at frequent intervals up to 20 days after dosing.

Samples of blood and urine were subjected to acid hydrolysis to convert all forms of haloxyfop into the acid form. Then the samples were analysed by GC with an electron-capture detector.

No adverse effects on health were reported in any segment of the study.

Orally administered racemic haloxyfop sodium salt was rapidly absorbed with a half-life of 0.9 h. Peak plasma concentrations (C_{max}) of haloxyfop of 1.4–2.2 µg/g were detected at 4–12 h after dosing. The removal from plasma into urine had a half-life of 6.3 days. Based on the amount of haloxyfop detected in urine, the volunteer in the pilot study absorbed and excreted 100% of the oral dose into the urine and the three volunteers in the second segment excreted 65–74% of the oral dose into the urine.

Racemic haloxyfop methyl ester was absorbed across skin slowly with a half-life of 36.5 h and peak plasma concentrations of haloxyfop of 0.1–0.2 µg/g were detected at 3 days after dosing. The haloxyfop was removed from plasma into the urine with a half-life of 5.8 days. An average of 2.4% of the haloxyfop applied to the skin was recovered in the urine.

The Meeting concluded that oral doses of haloxyfop methyl ester are rapidly and extensively absorbed. In contrast, dermal uptake of haloxyfop sodium is limited and slow. Urine was the principal route of excretion (65–100% of the excreted radiolabel) (Nolan et al., 1985).

A study in human volunteers was performed with Verdict Herbicide (a water-emulsifiable concentrate containing 25.7% haloxyfop-R methyl ester as its active ingredient). Detectable levels (limit of detection, LOD, and tissue not stated) were identified in only three out of eight volunteers after field application according to label instructions: diluting 5 US quarts (2.4 l) of the Verdict concentrate (containing 2.5 lbs [1.1 kg] of haloxyfop-R methyl ester) with 100 US gallons (378.5 l) of water and spraying 400 US gallons (1514 l) of diluted herbicide onto 20 acres of cropland using an open cab self-propelled or tractor-drawn boom spray. The skin exposure was assessed from patches placed under the clothing of the applicators. Inhalation exposure was estimated from air sampling. All of the urine passed in the 2 days after spraying was collected for biomonitoring analysis. Average skin exposure during mixing was estimated to be 959 µg per lb (2114 µg/kg) of active ingredient handled and during spraying it was 30 µg per lb (66 mg/kg) of active ingredient. The average inhaled dose of active ingredient during mixing was 9.1 µg/m^3 and during spraying it was 2.1 µg/m^3. The average absorbed dose of haloxyfop was 0.31 µg/kg bw, based on biomonitoring by urinary excretion. Detectable absorbed doses of haloxyfop were found in only three of the eight applicators with estimated absorbed doses ranging from < 0.04 to 1.4 µg/kg bw. No adverse health effects were reported in any of the applicators (Scortichini et al., 1987; Nolan et al., 1991)

1.2 Biotransformation

Mice

In a GLP-compliant study, groups of three male and three female mice were given radiolabelled (^{14}C label on the phenyl ring) racemic haloxyfop sodium salt as a single oral dose at approximately 10 (males) or 11 (females) mg/kg bw administered by gavage and samples of plasma were collected 6 h after dosing for HPLC profiling. Groups of three male and three female mice were given 18 (males) and 21 mg/kg bw (females) and urine was collected 24 h after dosing and bile was collected at 28 h after dosing for HPLC profiling. Haloxyfop was present in all samples analysed and was the only chemical species seen in plasma. The HPLC results for bile from males and females showed three major peaks. Three similar major peaks were seen in the HPLC results for urine from females (haloxyfop and two other substances), but only two peaks (including haloxyfop) were seen in males. Acid hydrolysis of the urine caused an increase in the haloxyfop peak, a reduction in the peaks for the other two substances and the appearance of a new more polar peak. The peak that was seen only in females corresponded to the glucuronide and disappeared completely after the acid hydrolysis. The Meeting concluded that, in the mouse, excretion of haloxyfop is in the form of unchanged haloxyfop and conjugates. Haloxyfop glucuronide appeared only in the urine of females. In addition, males and females eliminated another more polar conjugate in urine and bile (Smith et al., 1984).

Rats

As haloxyfop is chemically similar to 2-aryl-propionates that are known to undergo stereochemical inversion from *S*- to *R*-forms in several animal species, a study was conducted in rats to find out whether haloxyfop undergoes similar inversion. In a GLP-compliant study, groups of four male and four female Fischer 344 rats were given racemic [^{14}C]haloxyfop acid (purity, 98.7%; labelled in the phenyl moiety; radiochemical purity, 99.1%) as a single oral dose at 11 mg/kg bw by gavage. Urine and faeces were collected daily for 10 days after dosing. Analysis of urine and faeces showed that in females 79.4% of the radiolabel was excreted in urine and 10.5% in faeces within 10 days. In males, 16.9% was in the urine and 54.6% in the faeces. The *S*-haloxyfop underwent rapid and nearly complete inversion to the *R*-enantiomer in urine and faeces from males and females, with each daily collection of urine or faeces containing 0.2% or less of the administered dose in the form of the *S*-enatiomers. In males, the recovery of *S*-haloxyfop over the whole 10-day collection period was 0.3% of the administered dose in urine and 0.2% in faeces; while in females it was 0.1% in urine and 0.7% in faeces. The Meeting concluded that *S*-enantiomers of haloxyfop underwent rapid and almost complete stereochemical inversion to *R*-forms after oral dosing. (Bartels & Smith, 1988)

In a GLP-compliant study, groups of bile-duct-cannulated male and female Sprague-Dawley rats (numbers not stated) were given either ^{14}C-labelled racemic haloxyfop methyl ester (radiochemical purity, > 98%) or ^{14}C-labelled racemic haloxyfop acid (radiochemical purity, > 99%) as a single oral dose at 2.5 mg/kg bw by gavage. Both test materials were radiolabelled on the phenyl ring. Urine and bile were collected for about 48 h after dosing and plasma, erythrocytes and liver were sampled at 48 h. The samples were analysed by reverse-phase HPLC to determine whether haloxyfop methyl ester could be detected. For rats given haloxyfop methyl ester or haloxyfop acid, more than 97% of the radioactivity in plasma, erythrocytes and liver was found at the peak corresponding to haloxyfop acid and no haloxyfop methyl ester was detected in these samples. In urine of rats given haloxyfop methyl ester, 60% (males) and 89% (females) of the radioactivity resolved as haloxyfop acid, while the urine of those given haloxyfop acid had 74% (males) and 94% (females) of the radioactivity in the form of haloxyfop acid. The remainder of the radiolabel existed as unidentified metabolites

(one major and two minor). The amounts of haloxyfop acid in bile were 56% (males) and 48% (females) for those given haloxyfop methyl ester and 51% (males) and 55% (females) for those given haloxyfop acid. Only the major unidentified metabolite was detected in bile. Acid hydrolysis of the major unidentified metabolite in urine showed a 90% reversion to haloxyfop acid. It was also observed that, on standing urine or bile at room temperature for 24 h, the three unidentified metabolites were partly converted to haloxyfop acid. It is possible that the major metabolite was a conjugate of haloxyfop acid but attempts to identify it as a sulfate or glucuronide were inconclusive (Smith et al., 1982).

Samples of excreta and tissues were taken in the study of pharmacokinetics in rats given repeated doses of [^{14}C]haloxyfop-R methyl ester, as described in section 1.1. The amounts of radiolabel in the urine, faeces, plasma, tissues and residual carcass were measured. In addition, urine and faeces from selected intervals after dosing, liver, kidney and terminal plasma were analysed for metabolites by HPLC. The results showed that haloxyfop methyl ester was not present in the urine, plasma or tissues, but was present in faeces; 3.7% (males) and 8.2% (females) of the administered dose, being recovered in faeces as unchanged haloxyfop methyl ester (possibly representing unabsorbed material). Haloxyfop acid was the major metabolite found in urine (8.9% and 67.3% of administered dose in males and females, respectively), and it was the only metabolite found in plasma, liver and kidneys. Haloxyfop glucuronide was identified in the urine of females at 6.4% of the administered dose. In faeces, haloxyfop acid was the major metabolite, at 37.9% (males) and 13.4% (females) of the administered dose (Mendrala & Hansen, 2001).

In a GLP-compliant study that is described more fully in section 2.2, male Fischer rats were given diets containing racemic haloxyfop acid (purity, 99.5%) for a 4-week challenge period, followed by a 6-week recovery period. The doses given were 0 (control), 0.1 and 1.0 mg/kg bw per day. At 2-week intervals throughout the challenge and recovery periods, 7–10 rats from each group were killed, then blood was collected and pooled serum for each group was analysed for haloxyfop. Serum concentrations of haloxyfop at each sample time were approximately proportionate to the doses given. The plasma clearance half-life was 8 days for both doses (Herman et al., 1983).

Dogs

The analysis by GC and HPLC of the samples of tissues and excreta taken in the study of pharmacokinetics in dogs given an oral dose of racemic ^{14}C-haloxyfop acid (described in section 1.1) showed that the concentrations of haloxyfop in the plasma completely accounted for the amount of radiolabel that had been found in plasma. This suggested that unchanged haloxyfop was the main excretory product in dogs. Other metabolites appeared to be present in excreta. In faeces, most of the excretion was as haloxyfop, but there was a second unidentified metabolite that accounted for 8% of the radioactivity. In urine, haloxyfop was present, but a second major metabolite accounted for 50–68% of the radioactivity and was unaffected by acid hydrolysis. As most of the excretion was via the faeces, it might be concluded that haloxyfop was the major excretion product in the dog. However the presence of a second metabolite in urine is noted (Nolan et al., 1987).

Monkeys

The results of the study in cynomolgus monkeys that is described in section 1.1 suggested that oral doses of haloxyfop acid undergo little primary metabolism in cynomolgus monkeys (Gerbig et al., 1985).

2. Toxicological studies

2.1 Acute toxicity

(a) Systemic toxicity

(i) Oral administration

Mice

In a GLP-compliant study of acute oral toxicity, groups of five male and five female B6C3F_1 mice were given haloxyfop-R methyl ester (purity, 98.6%) as a single oral dose at 100, 500 or 1000 mg/kg bw by gavage in corn oil. The mice were observed for 15 days after dosing and were then killed for autopsy. The entire group of mice at the highest dose died within 3 days of treatment, after showing laboured breathing, lethargy, eyelid closure and perineal soiling. No gross pathology was seen in these mice at autopsy. In the group at the intermediate dose, all males and three of the females had thickened forestomachs (possibly due to irritancy), and all mices in this group gained little or no body weight. No treatment-related effects were seen in the mice given 100 mg/kg bw. The NOAEL was 100 mg/kg bw. The oral median lethal dose (LD_{50}) was 707 mg/kg bw (Mizell & Lomax, 1989a).

Rats

In a GLP-compliant study of acute oral toxicity, groups of five male and five female Fischer 344 rats were given haloxyfop-R methyl ester (purity, 98.6%) as a single oral dose at 100, 500 or 1000 mg/kg bw by gavage in corn oil. The rats were observed for 15 days after dosing and were then killed for autopsy. The entire group of rats at the highest dose and four males and one female from the group at the intermediate dose died within 8 days of treatment after showing lethargy and perineal soiling. At autopsy, several rats in the group at the highest dose showed signs of gastric irritation and had dark-red coloured urine in the bladder. No treatment-related effects were seen at 100 mg/kg bw. The NOAEL was 100 mg/kg bw. The oral LD_{50} was 300 mg/kg bw for males and 623 mg/kg bw for females (Mizell & Lomax, 1989b).

In a non-GLP-compliant study of acute oral toxicity, groups of six male and six female (except where indicated) Fischer 344 rats were given racemic haloxyfop acid (purity not reported) as a single oral dose at 250, 320 (males only), 400 (males only), 500, 1000 or 2000 mg/kg bw dissolved in a mixture of acetone and corn oil by gavage. The rats were observed for 15 days after dosing and were then killed for autopsy. All rats at 1000 or 2000 mg/kg bw died within 2 days and several rats from all of the groups at 320 mg/kg bw or more died during the 15-day observation period. The reported signs of toxicity were lethargy, rough coat, body tremors, hypersensitivity to stimuli, decreased appetite and dark exudate-staining around the eyes. Autopsies showed swollen livers with an accentuated lobular pattern in some rats. No treatment-related effects were seen in the rats at 250 mg/kg bw. The NOAEL was 250 mg/kg bw. The oral LD_{50} was 337 mg/kg bw for male rats and 545 mg/kg bw for females (Carreon et al., 1980).

Monkeys

In a GLP-compliant study of the acute oral toxicity of the sodium salt of racemic haloxyfop (purity, 99.6%), single doses at 40, 80 or 120 mg/kg bw or five daily doses at 20, 40 or 60 mg/kg bw per day were given to individual male cynomolgus monkeys by nasogastric intubation. The single dose of 40 mg/kg bw per day was well tolerated. Food consumption was decreased in the monkeys at 80 or 120 mg/kg bw, but returned to normal by 72 h after dosing. The monkey at 120 mg/kg bw also showed decreased physical activity for the 4 h after dosing. The repeated dose of 20 mg/kg bw per day

was well tolerated for the 5 days of treatment. The monkey at 40 mg/kg bw per day for 5 days showed a slight reduction in food consumption for 8 days and a concomitant reduction in body weight, but no effect was seen on physical activity or appearance. The treatment of the monkey at 60 mg/kg bw per day was stopped after 2 days, due to marked inappetance and emesis. This monkey continued to have decreased food consumption and had a concomitant loss of body weight; it showed general weakness, decreased activity and trembling with a progressive deterioration in physical condition. Gross and histopathological findings for the monkey given repeated doses of 60 mg/kg bw per day revealed lesions that were compatible with an infectious process and the pathology report attributed the progressive physical deterioration to an encephalopathy of unknown infectious etiology (Gerbig et al., 1985).

(ii) Dermal administration

Rats

The acute percutaneous toxicity of haloxyfop-R methyl ester was evaluated in a GLP-compliant study. Groups of five male and five female Fischer 344 rats were given a single dermal exposure at 2000 mg/kg bw on their clipped backs. The application site was kept under occlusive dressing for 24 h and was then washed. The rats were observed in the days after treatment and were killed at 15 days after treatment. No adverse effects were seen on mortality or body-weight gain. The only effects seen were lethargy and eyelid closure that were reported in two of the five males, but not in any females (Mizell et al., 1989).

(b) Dermal and ocular irritation

In a GLP-compliant study, 0.5 ml of undiluted haloxyfop-R methyl ester (purity, 95.7%) was applied to the clipped skin of three male and three female New Zealand White rabbits. The application site was kept under occlusive dressing for 4 h and was then wiped clean. Observations over the following 72 h indicated that haloxyfop-R methyl ester is not an irritant to skin (Mizell, 1989a).

The potential of undiluted haloxyfop-R methyl ester (purity, 98.6%) to cause eye irritation was investigated in a GLP-compliant study in three male and three female New Zealand White rabbits. Haloxyfop-R methyl ester did not cause eye irritation in this test (Mizell, 1989b)

(c) Dermal sensitization

The skin sensitization potential of haloxyfop-R methyl ester (purity, 98.6%) was tested in a GLP-compliant Buehler test in male Hartley guinea-pigs. The study used 20 guinea-pigs (10 receiving haloxyfop-R methyl ester and 10 positive controls. Haloxyfop-R methyl ester did not induce skin sensitization in this test. (Mizell, 1989c)

Undiluted haloxyfop-R methyl ester (purity not reported) was tested for skin sensitization potential in a Magnusson & Kligman maximization test, which was performed in accordance with GLP. Female Hartley guinea-pigs were used (10 receiving haloxyfop-R methyl ester and 5 controls). Haloxyfop-R methyl ester showed no skin sensitization potential in this test (Jones, 1994)

2.2 Short-term studies of toxicity

Mice

A 13-week feeding study studying mice was performed in accordance with GLP. Groups of 10 male and 10 female B6C3F$_1$ mice were fed diets containing racemic haloxyfop acid (purity,

96%) at a concentration designed to give doses of 0.002, 0.02, 0.2 and 2 mg/kg bw per day. A control group of 15 males and 15 females received basal diet. All mice were killed at the end of the treatment period and autopsied. Weights of brain, heart, liver, kidneys and testes were recorded. Terminal samples of blood were taken for haematology and clinical chemistry. A wide range of tissues from controls and mice at the highest dose were examined microscopically. For the other treatment groups, only the liver, gall bladder and kidneys were examined microscopically. There were no treatment-related deaths during the study and no abnormal behaviour or signs of toxicity were observed. There was reduced body weight in the females at 2 mg/kg bw per day when compared with concurrent controls from the sixth day of treatment onwards, but the body weights of all groups of males were unaffected. Food consumption was unaffected by treatment. There were no treatment-related effects on haematological parameters. Clinical chemistry results showed a statistically significant increase ($p < 0.05$) in serum alkaline phosphatase (AP) activity in the group of males at the highest dose (2 mg/kg bw per day) (22% greater than control values) and a small non-significant increase in this parameter in females (8% greater than control values). Absolute and relative weights of liver were also increased in male and female mice in the group at the highest dose and the livers appeared slightly enlarged and dark at autopsy. Microscopically, the centrilobular hepatocytes appeared enlarged in all males and 8 out of 10 females in the group at the highest dose and the cytoplasm of these cells was more eosinophilic and appeared more homogeneous than in controls. It is likely that the hepatocellular hyperplasia seen in this study was caused by a mode of action involving hepatocellular peroxisome proliferation that is not relevant to humans. The NOAEL was 0.2 mg/kg bw per day, on the basis of increased serum AP activity at 2 mg/kg bw per day (Gorzinski et al., 1982a).

In a 36-week feeding study that was performed in accordance with GLP, groups of 12 male and 12 female $B6C3F_1$ mice were fed diets containing racemic haloxyfop acid (purity, 96%) at a concentration designed to give dosaes of 0 (control group) or 2 mg/kg bw per day. An additional group of 10 males and 10 females was given a dose of 0.02 mg/kg bw per day. All mice were killed at the end of the treatment period and autopsied. Weights of brain, heart, liver, kidneys and testes were recorded. Terminal samples of blood were taken for haematology and clinical chemistry. Only the liver, gall bladder and kidneys were examined microscopically. Two males and one female from the group at the highest dose and one female from the group at the lowest dose died during the treatment period. No abnormal behaviour or signs of toxicity were observed and body weights and food consumption were unaffected by treatment. Haematology showed significantly decreased erythrocyte count in females at the highest dose but increased erythrocyte count and decreased erythrocyte volume fraction in the group at the lowest dose. These changes were not thought to be treatment-related. Clinical chemistry results showed increases in serum AP activity in males at the highest dose (105% greater than control values and statistically significant) and in females (8% greater than control values and non-significant). Absolute and relative weights of liver and kidney were increased in male and female mice in the group at the highest dose and the livers appeared slightly enlarged and dark at autopsy. Microscopically, the centrilobular hepatocytes appeared enlarged in all males and in 8 out of 10 females in the group at the highest dose. Also, the cytoplasm of these cells was more eosinophilic and appeared more homogeneous than in controls. One male mouse at the highest dose had a small focus of basophilic hepatocytes which the authors of the study suggested was consistent with the hepatocellular adenoma or type A nodule that is frequently seen in $B6C3F_1$ mice. It is likely that the hepatocellular hyperplasia seen in this study was caused by a mode of action involving hepatocellular peroxisome proliferation that is not relevant to humans. The kidneys of the males at the highest dose had decreased cytoplasmic vacuolation of the proximal convoluted tubule cells. The NOAEL was 0.02 mg/kg bw per day on the basis of increased serum AP activity and effects on the renal proximal convoluted tubules seen at 2 mg/kg bw per day (Gorzinski et al., 1982a).

Rats

In a 4-week probe study that was performed to GLP standards, groups of five male and five female Fischer 344 rats were given diets containing purified (purity, 99.99%) or technical-grade (purity, 99%) racemic haloxyfop acid at concentrations that gave doses of 0.01, 0.1, 1 or 10 mg/kg bw per day. A group of 10 males and 10 females was kept as controls. At the end of the treatment period, urine was collected for urine analysis (specific gravity, pH, glucose, protein, ketones, occult blood, bilirubin and urobilinogen) and blood was taken for haematology (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction, haemoglobin, platelets and erythrocyte morphology) and clinical chemistry (AP, alanine aminotransferase [ALT], glucose, blood urea nitrogen [BUN], total protein, albumin and globulin). All rats were then killed for autopsy. Weights of brain, liver, kidneys, heart, thymus and testes were recorded. A wide range of tissues from each rat were fixed, but only liver, kidneys, testes and epididymes were examined microscopically. The treatment did not affect mortality, behaviour, clinical signs and body weight or food consumption. Haematology showed that males at the highest dose, given either purified or technical-grade material, had statistically significant ($p < 0.05$) decreases in erythrocyte count, haemoglobin and erythrocyte volume fraction. Clinical chemistry showed statistically significant ($p < 0.05$) increases in serum AP activity, serum glucose and serum albumin in males given either technical or purified compound. Urine analysis parameters were unaffected by treatment. At autopsy, visibly enlarged livers were seen in all groups at the highest dose and in males at 1 mg/kg bw per day. Statistically significant increases ($p < 0.05$) in relative liver weight were seen in all groups at 10 mg/kg bw per day, and the males given a dose of 1 mg/kg bw per day of either test material showed a smaller increase that was not statistically significant. Microscopic examination of the liver showed swollen hepatocytes with eosinophilic cytoplasm that involved the entire hepatic lobule in males and females given 10 mg/kg bw per day of either test material, but was confined to the centrilobular area in males given 1 mg/kg bw per day of either material. Some of the males given 10 mg/kg bw per day of either material showed a slight decrease in the number of mature spermatozoa in the testes and epididymes. It was concluded that there was no difference in toxicity between the technical racemic haloxyfop acid and purified racemic haloxyfop acid. The NOAEL was 0.1 mg/kg bw per day on the basis of hepatic changes in males that were of no significance to humans. The NOAEL for effects that were relevant to the assessment of risk to humans was 1 mg/kg bw per day on the basis of changes in haematological and clinical chemistry parameters at 10 mg/kg bw per day (Gorzinski et al., 1982b).

In a GLP-compliant study, groups of 80 male Fischer 344 rats were given diets containing racemic haloxyfop acid (purity, 99.5%) for a 4-week challenge period, followed by a 6-week recovery period during which all rats received control diet. The doses given were 0 (control), 0.1 and 1.0 mg/kg bw per day. At 2-week intervals throughout the challenge and recovery periods, 7–10 rats from each group were killed, blood was collected and pooled serum for each group was analysed for haloxyfop. All rats were autopsied and liver weights were recorded. A wide range of tissues from each rat were fixed, but only liver was examined microscopically. The treatment had no effect on mortality, behaviour, clinical signs and body weight or food consumption. Serum concentrations of haloxyfop at each sample time were approximately proportionate to the doses given. The plasma clearance half-life was 8 days for both doses. Visibly enlarged livers were seen in the group of rats at the highest dose at both sacrifices during the challenge period, but no such effect was seen at any of the sacrifices during the recovery period (i.e. 2 weeks or more after dosing). Statistically significant increases in absolute and relative weights of liver were observed at both doses at the 2-week challenge sacrifice and in the group at the highest dose at the 4-week challenge sacrifice. Histopathological changes were seen in the livers of the group of rats at the highest dose at both challenge sacrifices, but no effects were seen at the lowest dose or in any group during the recovery period. The hepatic change reported

was centrilobular hepatocellular hyperplasia, accompanied by increased cytoplasmic eosinophilia. As the statistically significant increase in liver weight seen in the group at the lowest dose was not associated with any gross pathology or histopathology, this finding was regarded as fortuitous.

The NOAEL was 0.1 mg/kg bw per day on the basis of hepatocellular hyperplasia at 1.0 mg/kg bw per day. This effect was likely to be produced by a mode of action involving hepatocellular peroxisome proliferation and is of no relevance to humans. The NOAEL for effects of relevance to humans was the highest dose tested, 1 mg/kg bw per day (Herman et al., 1983).

In a GLP-compliant feeding study, groups of 10 male and 10 female Fischer 344 rats were fed diets containing haloxyfop-R acid (purity, 99.4%), receiving doses of 0 (control), 0.065, 0.2 or 2 mg/kg bw per day for 16 weeks. Additional groups of 10 males and females received doses of 0 or 2 mg/kg bw per day for 16 weeks, followed by a 4-week recovery period during which all rats received control diet. At the end of the study period for each group (with or without recovery), blood was collected from each rat for haematology (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction, haemoglobin and platelets) and clinical chemistry (glucose, BUN, creatinine, calcium, phosphorus, triglycerides, total protein, albumin, globulin, AP, ALT and aspartate aminotransferase (AST). Urine was collected at termination for urine analysis (specific gravity, pH, glucose, protein, ketones, occult blood, bilirubin and urobilinogen). All rats were killed for autopsy and weights of adrenals, brain, liver, kidneys, heart, thymus and testes or ovaries were recorded. A wide range of tissues from the groups of control rats and rats at the highest dose that were killed at the end of the challenge period were examined microscopically. For the other doses and the recovery groups, only a selection of tissues was examined, including liver, kidneys and lungs. The treatment had no effect on mortality, behaviour, clinical signs and body weight or food consumption. Small but statistically significant ($p < 0.05$) decreases in erythrocyte count, haemoglobin, and erythrocyte volume fraction were seen in males at the highest dose at the end of the treatment period and after the recovery period, but these effects were too small to be regarded as toxicologically significant. No adverse effects were seen in bone marrow. Clinical chemistry showed statistically significant ($p < 0.05$) increases in serum AP activity in males and females of the group at the highest dose after 16 weeks of treatment, but the effect was not seen after the 4-week recovery period. Serum cholesterol was significantly ($p < 0.05$) decreased at the end of the treatment period in males at 0.2 or 2 mg/kg bw per day and a small but non-significant decrease was seen in females at 2 mg/kg bw per day. The decreases in serum cholesterol were too small to be regarded as being toxicologically significant. By the end of the recovery period, serum cholesterol concentrations were back to normal. There were no effects on urine analysis parameters.

The treatment caused no gross lesions. Statistically significant ($p < 0.05$) increases in absolute and relative liver weights were seen in males and females of the group at the highest dose at the end of the treatment period and significant increases ($p < 0.05$) were also seen males given 0.2 mg/kg bw per day at the end of treatment and in males at the highest dose after the recovery period. Absolute and relative weights of testes were significantly ($p < 0.05$) decreased in males at the highest dose at the end of treatment and after the recovery period, but no histopathology was seen in the testes. In the livers of males and females of rats killed directly after treatment at 2 mg/kg bw per day, there was a slight hypertrophy of the centrilobular hepatocytes, accompanied by an increased eosinophilia of these cells. These hepatic changes were not apparent in rats killed after the recovery period. The hepatic changes were likely to be associated with a hepatocellular hypertrophy mediated by peroxisome proliferation and were not regarded as relevant to humans.

The NOAEL was 0.065 mg/kg bw per day on the basis of an increase in liver weight in males at 0.2 mg/kg bw per day. The NOAEL for effects relevant to humans was the highest dose tested, 2 mg/kg bw per day (Barna-Lloyd et al., 1989).

In a GLP-compliant study, groups of 15 male and 15 female Fischer 344 rats were fed diets containing racemic haloxyfop acid (purity, 96%), receiving doses of 0 (control), 0.002, 0.02, 0.2 or 2.0 mg/kg bw per day for 16 weeks. Blood samples were taken from 10 males and 10 females per group after 84, 99 and 106/7 days on test and haematology (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction and haemoglobin) was performed. Urine analysis (specific gravity, pH, glucose, protein, ketones, occult blood, bilirubin and urobilinogen) was performed at the end of the treatment period. All rats were killed for autopsy at the end of the treatment period and the following organs were weighed: brain, liver, kidneys, heart, thymus and testes. Clinical chemistry (glucose, AP, ALT, BUN, total protein, albumin and globulin) was performed on serum from blood taken at autopsy. Histopathology was performed on a wide range of tissues from 10 males and 10 females of the control group and group at the highest dose. For the other treatment groups, only the liver was examined microscopically.

The treatment had no effect on mortality, behaviour, clinical signs and body weight or food consumption. However, the growth of several of the rats in all groups was adversely affected by a viral infection. There were no treatment-related effects on haematological parameters. Serum AP activity was significantly ($p < 0.05$) increased by 24% in males given 2.0 mg/kg bw per day. Urine analysis showed no treatment-related effects. Absolute and relative liver weights were significantly ($p < 0.05$) increased in the group of females at the highest dose. In males, there were dose-related increases in absolute and relative liver weights, with the absolute weight being significant ($p < 0.05$) for the groups given 0.2 or 2.0 mg/kg bw per day and the increase in relative liver weight being significant ($p < 0.05$) at all doses tested. Small but significant ($p < 0.05$) increases in the relative weights of heart and kidneys were seen in females at 0.02 or 2.0 mg/kg bw per day. At autopsy, all males at the highest dose and two of the males at 0.2 mg/kg bw per day had visibly enlarged livers and several of the rats of either sex at 0.2 or 2.0 mg/kg bw per day had discoloured kidneys (darkened or greenish appearance). Microscopic examination showed hepatocellular hypertrophy in males at 0.2 or 2.0 mg/kg bw per day, with the effect being more severe at the highest dose. The hepatocytes of males and females at the highest dose had an increased degree of cytoplasmic homogeneity. No renal histopathology was seen.

The NOAEL was 0.02 mg/kg bw per day on the basis of hepatocellular hypertrophy (not relevant to humans) seen at 0.2 mg/kg bw per day or more. The small but statistically significant increases in the weights of liver, heart and kidneys that were seen at 0.02 mg/kg bw per day or less were not considered to be toxicologically significant as they were not associated with any effects on clinical chemistry or histopathology. The NOAEL for effects of relevance to humans was 0.2 mg/kg bw per day on the basis of the slightly increased serum AP activity at 2 mg/kg bw per day (Gorzinski et al., 1982c).

In a GLP-compliant study, groups of 12 male and 12 female Fischer 344 rats were fed diets providing racemic haloxyfop acid (purity, 96%) at a dose of 0 (control), 0.02 or 2.0 mg/kg bw per day for 37 weeks. Blood samples were taken from eight males and eight females per group after 258 days and haematology was performed (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction and haemoglobin). Urine analysis (specific gravity, pH, glucose, protein, ketones, occult blood, bilirubin and urobilinogen) was performed at the end of the treatment period. All rats were killed for autopsy at the end of the treatment period and the following organs were weighed: brain, liver, kidneys, heart, thymus and testes. Clinical chemistry (glucose, AP, ALT, BUN, total protein, albumin and globulin) was performed on serum from blood taken at autopsy. The liver and kidneys of all rats were examined microscopically. In addition, sections of kidney from five males in the control group and the group at the highest dose were treated with special stains (Zeihl Nielson acid fast, PAS, Gomori iron, Von Kossa calcium, Hall bilirubin and Dahl calcium) and were examined by microscope.

The treatment had no effect on mortality, behaviour, clinical signs and body weight or food consumption. However, the growth of several of the rats in all groups was adversely affected by a viral infection. There were no treatment-related effects on haematological parameters. Serum AP activity was significantly ($p < 0.05$) increased in males and females at 2.0 mg/kg bw per day. Urine analysis showed no treatment-related effects. Absolute and relative liver weights were significantly ($p < 0.05$) increased in groups of males and females at the highest dose. At autopsy, all males at the highest dose had enlarged livers and most (13 out of 15) also had darkened kidneys. Microscopy showed hepatocellular hyperplasia with cytoplasm of homogeneous appearance in males and females at the highest dose. In addition, there was a moderate increase in the amount of lipofuscin in renal tubular epithelial cells of males and (to a lesser extent) females at the highest dose. It was noted that this pigment can occur naturally in this strain of rat but has also been associated with exposure to peroxisome proliferators.

The NOAEL was 0.02 mg/kg bw per day on the basis of hepatocellular hypertrophy seen at 2.0 mg/kg bw per day. This effect was likely to have been produced by a mode of action involving hepatocellular hypertrophy that is not relevant to humans. The NOAEL for effects of relevance to humans was 0.02 mg/kg bw per day for increased serum AP activity at 2 mg/kg bw per day (Gorzinski et al., 1982c).

The dermal toxicity of the formulation EF-1400 has been evaluated in a GLP-compliant study in Fischer 344 rats. EF-1400 contained haloxyfop-R methyl ester at a concentration of 111 g/l. Groups of 10 males and 10 females were given EF-1400 as dermal doses (semi-occluded) at 0, 40, 200 or 1000 mg/kg bw per application, 6 h per day and 5 days per week for 13 weeks. The highest dose caused slight erythema, oedema, and/or scaling of the skin with very slight to slight hyperkeratosis and acanthosis. Dose-related decreases in erythrocyte count, haemoglobin and erythrocyte volume fraction were seen in males at 200 or 1000 mg/kg bw, and erythrocyte count was also decreased in males at 40 mg/kg bw. A treatment-related increase in the amount of hyaline droplets in the renal tubular epithelium was seen in males at all doses. Increased liver weight, increased serum AP activity and hepatocellular hypertrophy were seen in males at all doses and in females at the highest dose. Ultrastructural examination by electron microscope of centrilobular hepatocytes from rats at the highest dose showed increased numbers of peroxisomes in males but not females. No NOAEL was identified in this study (Stebbins et al., 2001).

Dogs

In a 5-week pilot feeding study, groups of two male and two female beagle dogs were given diets providing racemic haloxyfop acid (purity, 99.6%) at a dose of 0, 5, 15, or 45 mg/kg bw per day. The treatment at the highest dose caused gastroduodenal ulcers (males only), body-weight loss, decreased food consumption, decreases in serum cholesterol, erythrocyte volume fraction, erythrocyte count, haemoglobin and platelet count and increases in serum ALT, AP, bromosulfthalein retention and weights of liver and kidneys. At the intermediate dose there was reduced body-weight gain (males only), decreased serum cholesterol and increased liver weight (males only). No treatment-related effects were seen at the lowest dose. The NOAEL was 5 mg/kg bw per day (Barna-Lloyd et al., 1984).

In a GLP-compliant 13-week study, groups of four male and four female beagle dogs were given diets providing racemic haloxyfop acid (purity, 99.8%) at a dose of 0, 2, 5 or 20 mg/kg bw per day. Ophthalmology was performed on all dogs before treatment and at the end of the treatment period. Blood was taken for haematology (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction, haemoglobin and platelets) and clinical chemistry (glucose, AP, ALT, AST, BUN, total protein, albumin, globulin, Ca, Na, K, PO_4 and Cl)

before treatment and after 1, 2 and 3 months of treatment. The concentrations of several thyroid hormones (free-T3, free-T4, total-T3 and total-T4) were measured in the serum taken at 3 months. Urine was taken at 3 months for urine analysis (specific gravity, pH, glucose, protein, ketones, occult blood, bilirubin, urobilinogen, colour and microscopy of sediment). All dogs were killed at the end of the treatment period and autopsied and the weights of adrenals, brain, kidneys, liver, pituitary, thyroid (including parathyroid), ovaries and testes were recorded. An extensive range of tissues from all dogs in the control group and the group at the highest dose was examined by light microscopy, but the histopathology performed on the other dogs was limited to the following tissues: adrenals, epididymes, kidneys, liver, thyroid, parathyroid, pituitary, prostate, skin, testes, thymus and tonsils. Samples of liver were taken for measurement of hepatic fatty acid beta-oxidation (FAOX; by measuring cyanide-insensitive beta-oxidation of palmitoyl-CoA) in vitro and for electron microscopy.

No dogs died during the treatment period and no treatment-related clinical signs were observed. Body weights of males and females in the group at the highest dose became statistically significantly lower ($p < 0.05$) than the concurrent body weights of controls, but there was no effect on food consumption. No treatment-related effects were revealed by ophthalmoscopy. Small but statistically significant ($p < 0.05$) haematological changes were seen in the groups of dogs at the highest dose, for males and females: decreases in erythrocyte count, haemoglobin, erythrocyte volume fraction and platelet count. Clinical chemistry results showed that there were small dose-related decreases in serum cholesterol in males and females throughout the study that were statistically significant ($p < 0.05$) in all treated groups of males and in females at 5 and 20 mg/kg bw per day. The reduction in serum cholesterol concentrations was considered to be too small to be of toxicological significance. In addition, significant ($p < 0.05$) decreases in the serum concentrations of total triiodothyronine (T3), free T3 and total thyroxin (T4) were found in male and female dogs at 5 or 20 mg/kg bw per day. Absolute and relative weight of the thyroid/parathyroid was significantly ($p < 0.05$) decreased in males and females at 5 and 20 mg/kg bw per day and was associated with slight decreases in follicular size and hypertrophy of follicular epithelial cells. Relative kidney weights were increased in males and females in the group at the highest dose, but no renal pathology was seen and this effect was thought to be due to the loss of body fat by this group (as reflected in their lower body weight). Absolute liver weight, relative to controls, was significantly ($p < 0.05$) increased in males at 5 or 20 mg/kg bw per day and in females at 20 mg/kg bw per day; relative liver weight was significantly increased in males and females at 20 mg/kg bw per day. Light microscopy showed slight hepatocellular hypertrophy in males and females at 20 mg/kg bw per day and in females at 5 mg/kg bw per day that was shown to be due to increased stores of glycogen (vacuoles stained positive for glycogen but negative for fat). Electron microscopy showed no treatment-related changes. Hepatic activity of fatty acid beta-oxidation (FAOX) significantly increased in males and females at 5 and 20 mg/kg bw per day, but not at 2 mg/kg bw per day.

The NOAEL was 2 mg/kg bw per day on the basis of various effects seen at 5 and 20 mg/kg bw per day, including decreased serum thyroid hormone levels with associated morphological changes to the thyroid and induction of hepatic FAOX (Dietz et al., 1987).

In a GLP-compliant study, groups of six male and six female beagle dogs were given diets providing racemic haloxyfop acid (purity, 99.6%) at a dose of 0, 0.05, 0.5 or 5.0 mg/kg bw per day for 12 months. Blood was taken before treatment and after 1, 3, 6, 9 and 12 months of treatment for haematology (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction, haemoglobin, platelets, prothrombin time and reticulocyte count) and clinical chemistry (glucose, ALT, AST, AP, BUN, cholesterol, total protein, albumin, globulin, total bilirubin and triglycerides). In addition, liver function was tested by bromosulfthalein retention measurements at 6 and 12 months. Urine was collected for urine analysis before treatment and at 6 and 12 months.

All dogs were killed for autopsy at the end of the treatment period. The following organs were weighed: brain, heart, liver, kidneys, adrenals and ovaries or testes. Histopathology was performed on a wide range of tissues from dogs in the control group and the group at the highest dose. For the dogs in other treatment groups, histopathology was performed on only liver, kidney, bone marrow and bone.

The treatment had no effect on mortality, clinical signs, body-weight gain or food intake. Some of the measurements of erythrocyte volume fraction, erythrocyte count and haemoglobin were statistically significantly lower than concurrent control values, but comparison with historical control values showed that the concurrent control values were unusually high, so the effect was not considered to be treatment-related. Serum ALT and AST activities were significantly increased in the group of females receiving the highest dose at 12 months and ALT was also high in this group at 9 months. Serum cholesterol concentration was significantly decreased in males at the highest dose at 1, 6, 9 and 12 months. The reductions in concentrations of serum cholesterol and the increases in serum AST and ALT were too small to be of toxicological significance. Urine analysis parameters were unaffected by treatment. No treatment-related gross pathology was noted at autopsy and there was no effect on organ weights or histopathology. Bone marrow from all dogs appeared normal. The NOEL was 0.5 mg/kg bw per day (Barna-Lloyd et al., 1983 & 1984).

Liver samples from dogs in this study were later examined by electron microscopy. The results are summarized in section 2.7 (Barnard et al., 1986); no adverse effects of treatment were observed.

Monkeys

In a GLP-compliant 4-week study, groups of two male cynomolgus monkeys were given the sodium salt of racemic haloxyfop (purity, 99.6%) at a dose of 0, 5 or 20 mg/kg bw per day in distilled water by nasogastric intubation. An additional two monkeys were given a dose of 20 mg/kg bw per day for 4 weeks, followed by a 4-week recovery period. Data were collected on clinical appearance, behaviour, body weights, food consumption, haematology and clinical chemistry. At the end of the study, all monkeys were killed, autopsies were performed, organs were weighed and histopathological examinations were made of the major organs. Samples of liver and kidney were prepared for examination by electron microscopy, but only the liver samples were examined. The electron micrographs of the centrilobular area of livers were examined at 8000 × magnification for peroxisomal volume density, which was expressed as a percentage of cytoplasmic volume. The results showed no effects on mortality, clinical signs, body weight, food consumption, haematology, organ weights, gross pathology or histopathology. The only clinical chemistry parameter affected was serum cholesterol, which was slightly reduced at 20 mg/kg bw per day at the end of treatment, but was similar to control values in the monkeys that had a recovery period after treatment. In the absence of other effects, the reversible reduction in plasma cholesterol was not regarded as an adverse effect. Electron microscopy showed no effects on the appearance of liver tissue or on hepatocellular peroxisomal volume density. The NOAEL was the highest dose, 20 mg/kg bw per day (Gerbig et al., 1985).

The investigation of peroxisome proliferation in this study is also mentioned in section 2.8.

In a GLP-compliant 13-week study, groups of four male and four female cynomolgus monkeys were given racemic haloxyfop acid (purity, 99.8%) at a dose of 0, 2, 10 or 30 mg/kg bw per day dissolved in buffered water by nasogastric intubation. The monkeys were observed for clinical signs daily, body weights were measured weekly and eyes were examined with an ophthalmoscope at the beginning and end of the study. Blood samples were collected for haematology and clinical chemistry before the start of treatment (days −27 and −6) and during the treatment period (days 37, 66 and 92). Urine samples were collected for analysis before treatment (days −12 and −13) and towards the end of treatment (day 86). The monkeys were killed for autopsy at the end of 92 days of treatment.

Organs were weighed and histopathology was performed on major organs. Livers from the control group and the group at the highest dose were evaluated for electron microscopic and peroxisomal fatty acid beta-oxidation (FAOX) enzyme changes (by measuring beta-oxidation of palmitoyl-CoA in the presence of cyanide).

There was no mortality in the study and no treatment-related clinical signs were seen. The body weights of the groups of males and females at the highest dose were higher than those of the controls at the start of the treatment period and remained so throughout the study, but there was no effect on body-weight gain. Small but statistically significant decreases in erythrocyte count, haemoglobin and erythrocyte volume fraction were seen in males and females at 30 mg/kg bw per day, but smears of peripheral blood and bone marrow appeared normal, with differential cell counts similar to those for controls. Clinical chemistry showed statistically significant decreases in concentrations of serum triglycerides in males and females at all doses, but the values were not dose-related and were all within the range of values for historical controls. Urine analysis and ophthalmology revealed no adverse effects. Organ weights of liver and kidney were significantly increased in males and females of the group at the highest dose and kidney weight was also increased in males at 10 mg/kg bw per day. Thyroid weights were significantly decreased in males and females at the highest dose. At autopsy, thyroids were visibly smaller in the group of monkeys at the highest dose than in controls. The livers of the females at 10 mg/kg bw per day and males and females at 30 mg/kg bw per day had a pale appearance with an accentuated lobular pattern. Light microscopy showed slight hepatocellular hypertrophy with increased numbers of fatty vacuoles in monkeys at 10 or 30 mg/kg bw per day. In the thyroid there was decreased follicular size and hypertrophy of follicular epithelial cells in males and females at the highest dose and one female in the group at 10 mg/kg bw per day was similarly affected. No histopathological changes were seen in the kidneys. Ultrastructural evaluation by electron microscopy of centrilobular hepatocytes showed the livers of monkeys at the highest dose to have a slightly higher cytoplasmic lipid volume density and larger numbers of small fat droplets than controls. Peroxisomal volume densities were similar for controls and monkeys at the highest dose. Hepatic peroxisomal FAOX enzyme activity was not significantly different from that in controls at 30 mg/kg bw per day for males and females. The NOAEL was 2 mg/kg bw per day on the basis of changes to the liver and thyroid at 10 mg/kg bw per day or more (Yano et al., 1987).

The investigation of peroxisome proliferation in this study is also mentioned in section 2.7.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a GLP-compliant long-term study of combined toxicity and carcinogenicity, groups of 70 male and 70 female B6C3F$_1$ mice were fed diets providing racemic haloxyfop acid (purity, 99.6%) at a dose of 0, 0.03, 0.065 or 0.6 mg/kg bw per day. Satellite groups of 10 males and 10 females were killed at 6 and 12 months and the remaining 50 male and 50 female mice per dose were killed after 104 weeks of treatment. Terminal samples of blood were collected from mice in the main group and satellite groups for haematology and clinical chemistry, and the mice were autopsied with organ weights being recorded and microscopic examinations being performed.

There was no treatment-related effect on mortality, with 84–90% of males and 78–80% of females (non-satellite groups) surviving the scheduled 104 weeks. Body weights and feed consumption were unaffected by the treatment. No adverse haematological effects were reported at 6, 12 or 24 months. Clinical chemistry showed significantly elevated serum AP activity (16% increase relative to controls) in the group of males at the highest dose at the 12-month interim killing, but this parameter was not affected in females or in males at other time-points and there was no dose–response relationship. Other clinical chemistry parameters were unaffected. Also in males at the highest dose at 12 months, there was a statistically significant ($p < 0.05$) increase in relative liver weight of 14% compared

with that of controls (but no significant increase in absolute liver weight). Females of the group at the intermediate dose (0.065 mg/kg bw per day) had significantly reduced kidney weights (7% less than those of controls). Gross pathology on the mice killed at 24 months, showed a dose–response relationship to the number of mice with one or more liver masses/nodules, with incidences of 22, 30, 32 and 34% in males and of 12, 14, 26 and 26% in females at 0, 0.03, 0.065 and 0.6 mg/kg bw per day, respectively. Microscopic examinations of tissues showed a number of treatment-related lesions in the liver. The livers of the groups of males and females at the highest dose killed after 6, 12 or 24 months showed a slight increase in eosinophilia of the cytoplasm of hepatocytes in the centilobular region and a concomitant decrease in the amount of cytoplasmic vacuolation. Histopathological examination of the livers of the main groups of mice, that had been scheduled to be killed after 24 months, showed that there were some significant increases in the incidence of certain tumours in the group at the highest dose (see Table 3). The incidences of males with adenomas, males with adenomas or carcinomas and of females with carcinomas were all significantly increased, in comparison with controls. In addition, there was a significant positive dose-related trend towards increased incidence of adenomas or carcinomas. Only the increased incidence of carcinomas in females at the highest dose was above the range of data for historical controls from 11 earlier studies in the same strain of mice.

The NOAEL was 0.065 mg/kg bw per day on the basis of neoplastic and non-neoplastic hepatic changes at 0.6 mg/kg bw per day. The Meeting concluded that the results of this study indicated that high doses of haloxyfop could be tumorigenic to mice. The NOAEL for effects of relevance to humans was the highest dose tested, 0.6 mg/kg bw per day (Tollett et al., 1985).

Table 3. Liver tumours in mice in the main groups scheduled to be killed after 2 months

Finding	Dose (mg/kg bw per day)							
	Males				Females			
	0	0.03	0.065	0.6	0	0.03	0.065	0.6
Group size	50	50	50	50	50	50	50	50
Adenoma	8	10	15	19*	6	6	12	10
Carcinoma	5	8	7	9	1	3	2	8*
Adenoma or carcinoma	13	15	20	27*	7**	9	13	15

From Tollett et al. (1985)

* Value that is significantly different from the control value ($p < 0.05$).

** Significant dose–response relationship ($p < 0.05$).

Rats

In a GLP-compliant long-term study of combined toxicity and carcinogenicity, groups of 90 male and 90 female Fischer 344 rats were fed diets providing racemic haloxyfop acid (purity, 99.6%) at a dose of 0, 0.01, 0.03, 0.065 or 0.1 mg/kg bw per day for males, and 0, 0.01, 0.03, 0.065 or 1.0 mg/kg bw per day for females. Satellite groups of 20 males and females were killed at 6 and 12 months and the remaining 50 rats of each sex per dose were killed after 24 months of treatment. Data on clinical appearance, behaviour, body weights and feed consumption were recorded throughout the study. Haematology, clinical chemistry and urine analysis were conducted at 6 and 12 months for the satellite groups. For the main group, samples of blood and urine were taken for analysis at 18 and 24 months. Gross pathology, organ weights and histopathology were conducted at termination (6, 12 and 24 months).

At 2 years, survival of the various groups was 70–84% for males and 76–88% for females, with no dose–response relationship. Clinical signs, body weight and feed consumption were unaffected by

treatment. At 6 and 12 months, there were small and transient but statistically significant differences in some haematological parameters (erythrocyte count, haemoglobin, and erythrocyte volume fraction) from control values in various treated groups. The effects were not dose-related and there were no effects on these parameters seen at 18 and 24 months, so the haematological effects were not considered to be due to the treatment. Clinical chemistry showed significant increases in serum ALT in males at 0.065 and 0.1 mg/kg bw per day at 6 and 12 months. There were also significant decreases in serum globulin concentrations in males at 0.1 mg/kg bw per day at 6 months and in females at 1.0 mg/kg bw per day at 12 months. There was no effect on urine analysis parameters. No effects on organ weights were seen in the main groups of rats (24-month kill) or at the 12-month interim kill, but relative liver weights were increased at the 6-month interim kill in males at 0.1 mg/kg bw per day and males and females at 0.065 mg/kg bw per day.

The only treatment-related effect reported at autopsy was a dark or greenish discoloration of the renal cortex in the groups at the highest dose for males and females (0.1 mg/kg bw per day for males; 1.0 mg/kg bw per day for females). Histopathology revealed hepatocellular hypertrophy and cytoplasmic eosinophilia in the centrilobular region of the liver of the males and females at the highest dose at 6 months and in the females at the highest dose at 12 months. From 6 months onwards, an iron-containing pigment, that was suspected to be lipofuscin, was found in the proximal convoluted tubules of the kidneys of the group of rats at the highest dose. In the pancreas, adjacent to the islets of Langerhans of one male at the highest dose (0.1 mg/kg bw per day) and two females at the highest dose (1.0 mg/kg bw per day), there were focal accumulations of cells that appeared to be normal hepatocytes. The biological significance of this "ectopic hepatocellular tissue" is unclear. There was no evidence of an increased incidence of any benign or malignant neoplasm at any site.

The NOAEL was 0.065 mg/kg bw per day on the basis of hepatocellular hypertrophy in males at 0.1 mg/kg bw per day and in females at 1.0 mg/kg bw per day. The hepatocellular hypertrophy was likely to have been caused by a mode of action involving hepatocellular peroxisome proliferation that is not relevant to humans. The NOAEL for effect of relevance to humans was 0.1 mg/kg bw per day in males and 1 mg/kg bw per day in females, the highest doses tested (Tollett et al., 1983; Yano et al., 1984).

2.4 Genotoxicity

Haloxyfop-R methyl ester was tested for genotoxicity in a bacterial reverse mutation assay (Mecchi, 1999) and a mammalian cell cytogenetics assay (Linscombe et al., 1999). Haloxyfop-R acid was tested in assays for reverse mutation in bacteria (Gollapudi & Samson, 1989), cytogenetics in mammalian cells in vitro (Gollapudi & Linscombe, 1989) unscheduled DNA synthesis in vitro (McClintock & Gollapudi, 1989). Racemic haloxyfop acid was tested in assays for reverse mutation in bacteria (Domoradzki, 1981), gene mutation in mammalian cells in vitro (Myhr, 1982), cytogenetics in mammalian cells in vitro (Bootman et al., 1986), unscheduled DNA synthesis in vitro (Domoradzki, 1980) and micronucleus formation in rat bone marrow in vivo (Gollapudi et al., 1983). All of the tests gave negative results. Details are given in Table 4. Certificates of compliance with GLP were provided for all tests.

The Meeting concluded that haloxyfop-R methyl ester and haloxyfop were not genotoxic.

Table 4. Results of studies of genotoxicity with haloxyfop-R methyl ester and haloxyfop-R acid

End-point	Test object	Concentration/dose	Result	Reference
In vitro				
Haloxyfop-R methyl ester				
Reverse mutation	*Salmonella typhimurium* TA1535, TA1537, TA98 & TA100; *Escherichia coli* WP2*uvr*A	6.67–5000 µg/plate	Negative (±S9)	Mecchi (1999)
Cytogenetics	Rat lymphocytes	33.3–1000 µg/ml	Negative (± S9)	Linscombe et al. (1999)
Haloxyfop-R acid				
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA98 & TA100	5.0–5000 µg/plate	Negative (±S9)	Gollapudi & Samson (1989)
Cytogenetics	Rat lymphocytes	50–5000 µg/ml	Negative (±S9)	Gollapudi & Linscombe (1989)
Unscheduled DNA synthesis	Primary rat hepatocyte culture	5–5000 µg/ml	Negative	McClintock & Gollapudi (1989)
Haloxyfop acid				
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 & TA100	5.0–5000 µg/plate	Negative (±S9)	Domoradzki (1981)
Gene mutation	Chinese hamster ovary cells (CHO-K1), *Hprt* locus	25–2000 µg/ml	Negative (±S9)	Myhr (1982)
Cytogenetics	Human lymphocytes	100–1000 µg/ml	Negative (±S9)	Bootman et al. (1986)
Unscheduled DNA synthesis	Primary rat hepatocyte culture	0.3×10^{-9}–0.3×10^{-3} mol/l	Negative	Domoradzki (1980)
In vivo				
Haloxyfop acid				
Micronucleus formation	Sprague-Dawley rats (bone marrow polychromatic erythrocytes)	0, 30, 100 or 300 mg/kg bw as single oral dose	Negative (30 and 40 h harvests of bone marrow)	Gollapudi et al. (1983)

2.5 Reproductive toxicity

(a) Multigeneration studies

Racemic haloxyfop acid has been tested for effects on reproduction in a two-generation study and a three-generation study.

The two-generation study was performed in accordance with GLP. For the F_0 generation, groups of 30 male and 30 female Sprague-Dawley rats were given diets providing racemic haloxyfop acid (purity, 99.4%) at a dose of 0, 0.01, 0.065 or 1.0 mg/kg bw per day. After 8 weeks on the test diets, pairs of F_0 rats were mated to produce the F_{1a} generation. After weaning the F_{1a} litters, the F_0 rats were mated again to produce the F_{1b} generation. Groups of 30 F_{1b} weanling rats of each sex per dose were randomly chosen from the litters, and while being maintained on the same test diets as their parents they were mated twice to produce two further generations of rats: F_{2a} and F_{2b}. The rats were observed for general condition and behaviour and measurements were made of food intake and body weights. Data on reproductive parameters were collected, including duration of pregnancy, litter size, litter weight, pup weights and any gross abnormalities in the pups. All adults and 10 randomly selected weanlings of each sex from each group of the F_{1b} and F_{2b} generations were killed for autopsy. Tissues were stored for possible microscopic examination but were not looked at. The results showed no treatment-related effects on mortality, clinical signs, feed intake or body weights of the F_0 or F_1

adults. Some of the livers of the F_0 adults at 1.0 mg/kg bw per day appeared to be slightly enlarged, but the livers were not weighed or examined histopathologically, and the effect was not seen in the F_1 generation. Pup weights of the F_{1a} and F_{1b} rats in the group at 1.0 mg/kg bw per day were statistically significantly lower than those of the controls at age 14 and 21 days, and the body weights of the F_{1b} rats remained consistently less than hose of the controls as they were raised to adulthood. The body weights of the F_{2a} neonates were also decreased at age 21 days. Other litter parameters were not affected by the treatment and no grossly abnormal pups were seen. The NOAEL was 0.065 mg/kg bw per day on the basis of low pup body weights at 1.0 mg/kg bw per day (Nitschke et al., 1985).

The three-generation study was performed in accordance with GLP. For the F_0 generation, groups of 30 male and 30 female Fischer 344 rats were fed diets designed to provide racemic haloxyfop acid (purity, 99.6%) at a dose of 0, 0.005, 0.05 or 1.0 mg/kg bw per day. After 102 days on the test diets, the rats were mated to produce F_{1a} litters, which were killed for autopsy after weaning. The F_0 rats were rested for 1 week after weaning of their litters and then mated again to produce the F_{1b} litters. After all the F_{1b} litters were weaned, the F_0 rats were killed for autopsy. For each sex at each dose, 30 rats of the F_{1b} generation were randomly selected for mating (after 137 days on test diets) to produce three litters of F_2 generation rats (F_{2a}, F_{2b} and F_{2c}). After weaning of the F_{2c} litters, all of the F_{1b} parent rats were killed for autopsy and histopathology. For males and females of each test group, 10 of the surplus F_{2b} rats that were not chosen for mating were killed for autopsy, and five of each sex per group of these were used for histopathology. The first litter from the F_{1a} matings (ie. the F_{2a} litter) was weaned and then killed at day 28 of life, with autopsies being performed on 10 rats of each sex per group. For each sex at each dose, 30 rats of the F_{2b} generation were randomly selected for mating with one another (after 64 days on test diets) to produce two litters of F_3 generation rats (F_{3a} and F_{3b}). All the F_{2b} adults were killed for necropsy (no histology) after the F_{3b} litters were weaned. The F_{3b} pups were killed at day 28 of life and 30 males and 30 females per group were randomly chosen for necropsy (histopathology on five of these); the F_{3a} pups were not autopsied. From the F_{2c} rats, 30 males and 30 females per group were fed the test diets for 61 days and then were mated with untreated males and untreated females. The mated females (treated F_{2c} females that had been mated with untreated males and untreated females that had been mated with treated F_{2c} males) were killed at mid-gestation and their uterine contents were examined.

There were no treatment-related effects on mortality, clinical signs, body weight or feed consumption for any of the generations of adult rats. Most of the indices of reproductive performance, including number of live pups born, gestational survival index and sex ratio of pups, were unaffected by treatment at all generations. The fertility index of the treated rats was less than the control value for the second and third matings of the F_{1b} adults (see Table 5). Although the effect was not dose-related, it was statistically significant for the groups at the intermediate (0.05 mg/kg bw per day) and highest (1.0 mg/kg bw per day) dose of the second mating of the F_{1b} rats (to produce the F_{2b} pups) and group at the highest dose (1.0 mg/kg bw per day) for the third mating of the F_{1b} rats (to produce the F_{2c} pups). The authors of the study did not consider this effect to be treatment-related as it was not dose-related and not seen in the other two generations. Although there was no treatment-related effect on pup birth weight, the body weights of F_{1b} and F_{2a} pups of the group at the highest dose (1.0 mg/kg bw per day) were significantly ($p < 0.05$) less than concurrent control values on most days during weaning and up to termination at 28 days. No such effect on pup body weight was seen for the F_{1a}, F_{2b} or F_{2c} pups. Autopsies on the adults showed some changes in organ weights at the highest dose of 1 mg/kg bw per day: liver weights were increased while kidney and testes weights were decreased. Histopathology showed some changes to the liver (hepatocellular hypertrophy with centrilobular eosinophilia and decreased intracellular lipid) and kidneys (pigment granules in the epithelium of proximal convoluted tubules), but no adverse histopathology was seen in the testes. The authors considered the effect on the liver to be a treatment-related adverse effect, but not the effects on the kidneys and testes. Autopsies on pups showed increased liver weight in F_{1b}, F_{2a} and F_{2b} pups, but with no corresponding histopathology. The Meeting concluded that there was no convincing evidence that

doses of up to 1.0 mg/kg bw per day caused any adverse effect on reproduction in Fischer 344 rats. The NOAEL was 0.05 mg/kg bw per day on the basis of hepatocellular hypertrophy at 1.0 mg/kg bw per day. This effect was not regarded as being relevant to humans. The NOAEL for effects of relevance to humans was the highest dose tested, 1 mg/kg bw per day (Jeffries et al., 1985).

Table 5: Fertility index of F344 rats fed diets containing racemic haloxyfop acid

Generation		Fertility index (%)			
Adults mated	Pups produced	0 mg/kg bw per day	0.005 mg/kg bw per day	0.05 mg/kg bw per day	1.0 mg/kg bw per day
F_0	F_{1a}	83	93	93	97
F_0	F_{1b}	87	87	97	93
F_{1b}	F_{2a}	63	57	67	53
F_{1b}	F_{2b}	73	50	47*	47*
F_{1b}	F_{2c}	70	43	50	40*
F_{2b}	F_{3a}	53	63	53	67
F_{2b}	F_{3b}	77	80	83	90
F_{2c}	Cross-mating	90	90	83	93

From Jeffries et al. (1985)

* Statistically significantly different ($p < 0.05$) from the value for untreated controls (0 mg/kg bw per day).

(b) Developmental toxicity

Rats

In a GLP-compliant study of developmental toxicity, groups of 30 mated female Fischer 344 rats were given oral doses of the sodium salt of racemic haloxyfop (purity, 99.7%) at 0, 0.1, 1.0 or 7.5 mg/kg bw per day by gavage on days 6 to 15 of gestation. On day 21 of gestation, all surviving dams were killed and autopsied. Livers were weighed and liver plus other tissues were fixed, but histopathology was not performed. Uteri were examined for number of live and dead fetuses, position of fetuses, number and position of resorption sites, number of corpora lutea, sex, body weight and length of fetuses, and any overt fetal malformations. Apparent non-pregnancy was confirmed by staining the uterus with sodium sulfide. At least half the fetuses from each litter were examined by microdissection for evidence of visceral malformations. The remaining fetuses were examined for skeletal malformations. No maternal deaths occurred during the study and no clinical signs were seen. At the highest dose (7.5 mg/kg bw per day), there was decreased feed intake and decreased body-weight gain on days 6 to 8 of gestation, low body weight when compared with that of controls on days 16 to 21, and increased intake of water on days 12 to 20. There were no effects on mortality, clinical signs, or liver weight of the dams. Pregnancy rate was not affected. Litter data showed no effects on number of resorbed implantations, pre-implantation loss, litter size, sex ratio, fetal weight or fetal length. There was no effect of treatment on the numbers of any visceral malformations in fetuses. The only skeletal effect was a significantly increased incidence ($p < 0.05$) of fetuses with incompletely ossified centra of thoracic vertebrae in the group at the highest dose (6% fetuses; 33% litters) when compared with the rate in controls (fetuses, 1%; litters, 8%). The Meeting concluded that no embryotoxicity or teratogenicity was seen in this study, and that the NOAEL was 1.0 mg/kg bw per day on the basis of delayed ossification in fetuses at 7.5 mg/kg bw per day (Hayes et al., 1983).

Rabbits

In a GLP-compliant study of developmental toxicity, groups of 30 mated female New Zealand White rabbits were given the sodium salt of racemic haloxyfop (purity, 99.7%) as an oral dose at 0,

1.0, 7.5 or 20 mg/kg bw per day by gavage on days 6 to 18 of gestation. On day 29 of gestation, all surviving dams were killed and autopsied. Livers were weighed and liver plus other tissues were fixed but histopathology was not performed. Uteri were examined for number of live and dead fetuses, position of fetuses, number and position of resorption sites, number of corpora lutea, sex, body weight and length of fetuses, and any overt fetal malformations. Apparent non-pregnancy was confirmed by staining the uterus with sodium sulfide. At least half the fetuses from each litter were examined by microdissection for evidence of visceral malformations. The remaining fetuses were examined for skeletal malformations. Seven maternal deaths occurred during the study: one in the group at the lowest dose, one in the group at the intermediate dose and five in the group at the highest dose. Autopsies showed the rabbit at the intermediate dose died of acute pneumonia that may have been due to an intubation error, pleuritis and constrictive pericarditis were seen in the rabbit at the lowest dose and pleuritis and pneumonia were seen in one of the rabbits at the highest dose. The cause of death was not identified for the other four rabbits at the highest dose. No clinical signs were reported. Body weight and liver weight were unaffected by treatment. Pregnancy rate was not affected. There was a significant ($p < 0.05$) increase in the number of resorbed implantations in the group at the highest dose (22% of implantations, affecting 79% of litters) compared with controls (9% of implantations, affecting 30% of litters). Other litter parameters were unaffected by treatment. Atypically, no external or soft tissue malformations were seen in fetuses from the control group, and although a few external and soft tissue malformations were recorded for the treated groups, the incidences were mostly well within the range of incidences among historical controls for the laboratory. The exceptions were the findings of one fetus at the intermediate dose and one fetus at the highest dose with microphthalmia, for which there had been no reports in the data for historical controls. As only single fetuses were affected at these doses, the authors suggested that this finding was incidental. Skeletal findings showed a significantly ($p <0.05$) increased number of litters containing fetuses with a crooked hyoid bone in the group at the highest dose (19%) compared with controls (4%). The finding was not significant when calculated on the basis of the number of fetuses affected rather than the number of litters. The NOAEL was 7.5 mg/kg bw per day on the basis of fetotoxicity (crooked hyoid bone) and unexplained maternal mortality at 20 mg/kg bw per day (Hayes et al., 1983).

As a result of concern over the relevance of the microphthalmia that was seen in some test groups in the earlier study of developmental toxicity in rabbits, a repeat study was performed. The same batch of sodium salt of racemic haloxyfop was used. Analysis of this test material showed it to be 99.6% pure. The study was GLP-compliant. Groups of 25–30 mated female New Zealand White rabbits were given the test material as an oral dose at 0, 3.0, 7.5 or 15 mg/kg bw per day by gavage on days 6 to 18 of gestation. On day 29 of gestation, all surviving dams were killed and autopsied. Livers were weighed and liver plus other tissues were fixed but histopathology was not performed. Uteri were examined for number of live and dead fetuses, position of fetuses, number and position of resorption sites, number of corpora lutea, sex, body weight and length of fetuses, and any overt fetal malformations. Apparent non-pregnancy was confirmed by staining the uterus with sodium sulfide. At least half the fetuses from each litter were examined by microdissection for evidence of visceral malformations. The remaining fetuses were examined for skeletal malformations. Five maternal deaths occurred during the study: one in the group at the lowest dose and four in the group at the highest dose. Two of the deaths in the group at the highest dose appeared to be due to intubation error, but the cause of death of the other rabbits was not clear. No clinical signs were reported. Body weight and liver weight were unaffected by treatment. Pregnancy rate was not affected. There was a statistically significantly increased rate of resorptions in the group at the lowest dose (32% of implantations affecting 78% of litters) compared with controls (10% of implantations; 63% of litters), but no effect was apparent at higher doses so this was considered to be an incidental finding. Other litter parameters were unaffected. Examinations of the fetuses showed no cases of microphthalmia. There was no significant effect on the incidence of crooked hyoid bone at any dose and the incidence of fetuses with

delayed ossification in the hyoid bone was significantly decreased in the group at the lowest (29%) and the intermediate (27%) dose compared with controls (46%). There were no treatment-related adverse effects on the incidences of any external, soft tissue or skeletal malformations.

The NOAEL was 7.5 mg/kg bw per day on the basis of the unexplained maternal mortality at 15 mg/kg bw per day. The Meeting concluded that no developmental toxicity was seen at any dose tested (Hanley et al., 1985).

The increased incidence of litters with crooked hyoid bone that was found in the initial study of developmental toxicity in rabbits was not found in the repeat study. As the effect was not reflected in an increased incidence of fetuses with crooked hyoid, and the effect was seen at only one maternally toxic dose and as crooked hyoid is regarded as a minor variation rather than a more serious effect, the Meeting concluded that there was no convincing evidence that the sodium salt of racemic haloxyfop could cause developmental toxicity (including fetotoxicity) in rabbits.

2.6 Special studies: hepatic peroxisome proliferation

In addition to observations on the effects on hepatic peroxisomes incorporated into some of the conventional studies of toxicity in non-rodents that are mentioned above, several separate investigations of effects on hepatocellular peroxisomes and the reversibility of these effects have been conducted in vitro and in rodents, dogs, and monkeys.

The effects of racemic haloxyfop acid (purity, 98.8%) on peroxisome proliferation, replicative DNA synthesis at the S-phase of the cell cycle and apoptosis were investigated in a primary culture of hepatocytes derived from young male $B6C3F_1$ mice. The study was not GLP-compliant, as the laboratory was not GLP-certified, but the study was performed in accordance with most of the concepts and principles of GLP. Haloxyfop acid was used at a concentration of 0, 10, 30, 100, 300 and 1000 µmol/l dissolved in DMSO. The positive controls used were: epidermal growth factor (EGF) at 25 ng/ml, for replicative DNA synthesis; transforming growth factor beta-1 ($TGF\beta_1$) at 5 ng/ml, for apoptosis; and WY14,643 at 100 µmol/l, for replicative DNA synthesis, apoptosis and peroxisome proliferation. The amount of peroxisome proliferation was estimated by measuring the amount of cyanide-insensitive oxidation of palmitoyl CoA (FAOX). The results showed highly significant ($p < 0.001$) increases in the rate of oxidation of palmitoyl CoA at doses of 30 µmol/l or more (indicating peroxisome proliferation), significantly decreased ($p < 0.05$) replicative DNA synthesis at 30 µmol/l or more, and significantly increased ($p < 0.05$) numbers of hepatocytes undergoing apoptosis at doses of 10 µmol/l or more. Most of the positive controls gave the expected results, except that the increase in apoptosis with WY14,643 was not statistically significant. The Meeting concluded that racemic haloxyfop acid caused an increase in peroxisome proliferation, a decrease in replicative DNA synthesis and an increase in apoptosis in cultured mouse hepatocytes. This was not the anticipated result, as the authors had expected to find increases in all three parameters (Elcombe, 2002a).

A study with similar protocol to the studies in mouse or human hepatocytes in vitro was performed in guinea-pig hepatocytes using the same concentrations of the same batch of racemic haloxyfop acid. In this study there were significant ($p < 0.01$) increases in peroxisome proliferation at concentrations of 10, 100, 300 and 1000 µmol/l, highly significant ($p < 0.001$) dose-related decreases in replicative DNA synthesis at all doses (10 µmol/l or more), and highly significant ($p < 0.001$) increases in the number of cells undergoing apoptosis at all doses (10 µmol/l or more). This time the material used as a positive control WY14,643 did not give significant changes in either replicative DNA synthesis or apoptosis, although the other positive controls gave the expected significant increases in the corresponding parameters. The Meeting concluded that racemic haloxyfop acid caused an

increase in peroxisome proliferation, a decrease in replicative DNA synthesis and an increase in apoptosis in cultured guinea-pig hepatocytes (Elcombe, 2002b).

A study with similar protocol to the studies in mouse and guinea-pig hepatocytes in vitro was performed in human hepatocytes using the same batch of racemic haloxyfop acid. Concentrations of 0, 30, 300 and 1000 µmol/l were used. Samples of human liver were obtained from three patients undergoing resections for the removal of tumours. The liver cultures from each donor were tested separately. This time, WY14,643 was used as a positive control for induction of peroxisome proliferation only and not for replicative DNA synthesis or apoptosis. The results showed no consistent effect of racemic haloxyfop acid on peroxisome proliferation, although there was a significant increase for one donor at 30 µmol/l and a significant decrease for another donor at 100 µmol/l. The positive control (WY14,643 at 100 µmol/l) had no effect on peroxisome proliferation in cells from any of the donors, suggesting that human hepatocytes are not responsive (or, at least, not as responsive as mouse or guinea-pig hepatocytes) to peroxisome proliferators. There was a dose-related decrease in replicative DNA synthesis that was significant ($p < 0.05$) in all three sets of cells from different donors at 300 µmol/l or more, and at 30 µmol/l there was a significant decrease with the cell culture from one of the donors. The positive control (EGF at 25 ng/ml) caused a highly significant ($p < 0.001$) increase in replicative DNA synthesis in all three sets of cells. There were significant ($p < 0.05$) increases in apoptosis in all three donors at concentrations of 300 µmol/l or more and in two of the donors at 30 µmol/l, and significant increases were seen in cells from all three donors in response to the positive-control material ($TGF\beta_1$ at 5 ng/ml) (Elcombe, 2002c).

The results of in-vitro studies in hepatocytes from different species showed that human hepatocytes responded to racemic haloxyfop acid in a different way to hepatocytes from the other two species, in that they did not show the increase in peroxisome proliferation that was seen in mice and guinea-pigs (Table 6). The hepatocytes from all three species showed a decrease in replicative DNA synthesis and an increase in the number of cells undergoing apoptosis. The direction of the effect on replicative DNA synthesis was opposite to the direction of the response to the positive control (EGF). The finding of decreased replicative DNA synthesis at S-phase was unexpected and no explanation for it was available.

Table 6. Effects of haloxyfop acid on hepatocytes from different species in vitro

Effect	Species		
	Mouse	Guinea-pig	Human
Haloxyfop acid			
Peroxisome proliferation	+	+	0
Replicative DNA synthesis	−	−	−
Number of cells undergoing apoptosis	+	+	+
Positive controls			
Peroxisome proliferation (WY14,643)	+	+	0
Replicative DNA synthesis (EGF)	+	+	+
Number of cells undergoing apoptosis ($TGF\beta_1$)	+	+	+

From Elcombe (2002a), (2002b) & (2002c)

EGF, epidermal growth factor; $TGF\beta_1$, transforming growth factor beta-1.

\+ Increase in this parameter when compared with untreated controls.

0 No consistent increase or decrease (similar to untreated control).

− Decrease in this parameter when compared with untreated controls.

Mice

In a GLP-compliant study, groups of 25 male and 25 female B6C3F$_1$ mice were given diets providing racemic haloxyfop acid (purity, > 99%) at a dose of 0, 0.1, 0.5, 1.0 or 10 mg/kg bw per day for 4 weeks. This was followed by a 2-week or 4-week recovery period. Possible effects that might have been related to peroxisome proliferation were monitored at the end of the 4-week treatment period and after the 2-week and 4-week recovery periods. These possible effects were monitored by measuring liver weights, light and electron microscopy of the liver, and hepatic enzyme activities of FAOX, catalase, glycerophosphate dehydrogenase (GPD) and carnitine acyl transferase (CAT). The treatment groups were divided into subgroups of three males and three females for light and electron microscopy and of eight (end of treatment) or three to four (recovery periods) males and females for assays for enzyme activity.

No clinical signs or effects on body weight were reported for any group.

Immediately after the treatment period, liver weights were greatly increased (75–90%) in males and females at 10 mg/kg bw per day. Light microscopy showed changes in all treated groups, including hepatocellular hypertrophy and/or increased cytoplasmic eosinophilia. The changes were seen throughout the liver. Electron microscopy showed increased peroxisome volume densities at 10 mg/kg bw per day in males (8-fold) and females (14-fold). Both the size and number of peroxisomes had increased. Induction of FAOX, relative to controls, was seen at 10 mg/kg bw per day in males (237%) and females (157%). Catalase was induced in males and females at 10 mg/kg bw per day (106%). CAT was induced in males and females at 1.0 (230%) and 10 mg/kg bw per day (1400%) and in females at 0.5 mg/kg bw per day (70%). Male and female mice at 10 mg/kg bw per day had a 19–38% increased activity of GPD, relative to controls.

After the recovery periods, the effect on liver weights was less marked with significant increases seen only at the highest dose for males and females at 2 and 4 weeks after dosing. Hepatocellular hypertrophy and eosinophilia were seen only at 10 mg/kg bw per day at 2 weeks recovery (males and females), and were not seen in any group after 4 weeks recovery. Electron microscopy was not performed on the mice allowed a recovery period. FAOX and CAT were induced (up to 200%) only in the group at the highest dose (both sexes) at the 2-week recovery. No induction of GPD or catalase was seen in any group after the 2-week or 4-week recovery periods.

A NOAEL was not identified for this study as hepatocellular hypertrophy was seen at all doses tested. Hepatocellular peroxisome proliferation was demonstrated at 10 mg/kg bw per day. Biochemical changes associated with the peroxisome proliferation were seen at doses of 0.5 mg/kg bw per day or greater (Stott et al., 1985a & 1985b)

In a study that complied with GLP, groups of 20 female B6C3F$_1$ mice were given oral doses of haloxyfop acid (presumably racemic; purity, > 98.5%) at a dose of 0 (vehicle control) or 0.6 mg/kg bw per day by gavage for 14 days. A group of five mice serving as positive controls was given clofibric acid at a dose of 200 mg/kg bw per day for 14 days. All rats were killed at the end of the treatment period. The livers from the mice were weighed and analysed for enzyme activity of peroxisome acyl-CoA (ACO). RNA was isolated and pooled RNA from five mice per group was used for measurement of gene expression levels for selected genes (ACO, CYP4A10, CTE-I, Bcl-2, Bak-1 and *c-myc*), using real-time polymerase chain reaction (RT-PCR) analysis. Treatment with haloxyfop acid caused significant increases in absolute and relative liver weights, a 3-fold increase in ACO activity and increased expression of the ACO (2.4-fold), CYP4A10 (13-fold) and CTE-I (15-fold) genes. These up-regulated genes had previously been shown to be associated with peroxisome proliferation. A slight increase was seen in transcription of *c-myc* (1.5-fold). There was no clear effect on markers of apoptosis, as increased transcription was seen for both the anti-apoptotic marker Bcl-2 (2.2-fold) and the pro-apoptotic marker Bak-1 (1.4-fold). A similar pattern of induced gene expression (although a smaller response) was seen in the positive controls that were given clofibric acid at 200 mg/kg bw

per day. The Meeting concluded that treatment with haloxyfop acid at 0.6 mg/kg bw per day for 14 days caused biochemical changes that are associated with peroxisome proliferation in the livers of the treated mice (Charles et al., 2002).

Rats

In a study that complied with GLP, groups of 25 male and 25 female Fischer 344 rats were given diets containing racemic haloxyfop acid (purity, > 99%) at a dose of 0, 0.1, 0.5, 1.0 or 10 mg/kg bw per day for 4 weeks. This was followed by a 2-week or 4-week recovery period. Possible effects that might be related to peroxisome proliferation were monitored at the end of the 4-week treatment period and after the 2-week and 4-week recovery periods. The parameters related to peroxisome proliferation that were measured were liver weights, light and electron microscopy of the liver, and enzyme activities of FAOX, catalase, GPD and CAT. The treatment groups were divided into subgroups of three males and three females for light and electron microscopy and of eight (end of treatment) or three to four (recovery periods) of each sex for enzyme assays.

No clinical signs were reported for any group. In the males at 10 mg/kg bw per day, body weight was reduced by 4% relative to controls.

Immediately after the treatment period, relative liver weights were significantly increased in males at 1.0 or 10 mg/kg bw per day, but were significantly decreased in females at 10 mg/kg bw per day, relative to controls. Light microscopy showed changes in all treated groups, including hepatocellular hypertrophy and/or increased cytoplasmic eosinophilia. The changes were seen throughout the liver in males at the highest dose, but were restricted to the centrilobular area in females at all doses and in males at doses of up to 1.0 mg/kg bw per day. Electron microscopy showed increased peroxisome volume density at 10 mg/kg bw per day in males (12-fold), but not in females. Both the size and number of peroxisomes had increased. Induction of FAOX, relative to controls, was seen at 10 mg/kg bw per day in males (393%) and females (109%). Smaller increases were seen in males at 0.5 (60%) and 1.0 mg/kg bw per day (194%). Catalase was induced in males at 1.0 (45%) and 10 mg/kg bw per day (74%), but catalase activity was only slightly increased (34%) at 10 mg/kg bw per day in females. CAT was induced in males at 0.5 (260%), 1.0 (360%) and 10 mg/kg bw per day (2200%) and in females at 10 mg/kg bw per day (450%). In male rats at 10 mg/kg bw per day, hepatic GPD was statistically significantly increased, with an activity that was 52% greater than that in males in the control group. Hepatic GPD was not significantly increased in females and there was no dose–response relationship, although hepatic GPD activities in groups treated with haloxyfop were 19–36% greater than those in females in the control group.

After the recovery periods, the effect on liver weights was less marked, with significant increases seen only in the males at the highest dose at 2 and 4 weeks after dosing. Hepatocellular hypertrophy and eosinophilia were seen only in males at 1.0 or 10 mg/kg bw per day at 2 weeks recovery and only in males at the highest dose (10 mg/kg bw per day) at 4 weeks recovery. Electron microscopy was not performed for rats allowed a recovery period. Induction of FAOX (up to 200%), CAT (up to 200%) and GPD (71%) was seen only in the group of males at the highest dose after a 2-week recovery period. No induction of catalase was seen in any group after the 2-week or 4-week recovery periods.

A NOAEL was not identified for this study as hepatocellular hypertrophy was seen at all doses tested. Hepatocellular peroxisome proliferation was demonstrated at 10 mg/kg bw per day. Biochemical changes associated with peroxisome proliferation were seen at doses of 0.5 mg/kg bw per day or greater (Stott et al., 1985a & 1985b).

A 13-week study of dermal toxicity with the formulation EF-1400 (haloxyfop-R methyl ester at 111 g/l) was performed in Fischer 344 rats at doses of 0, 40, 200 or 1000 mg/kg bw per application, 6 h per day and 5 days per week. The study is described more fully in Section 2.3. Increased liver weight, increased serum AP activity and hepatocellular hypertrophy were seen in males at all doses

and in females at the highest dose. Ultrastructural examination by electron microscope of centrilobular hepatocytes from rats at the highest dose showed increased numbers of peroxisomes in males but not females. No NOAEL was identified for this study (Stebbins et al., 2001).

Guinea-pigs

Groups of five Dunkin-Hartley guinea-pigs were given racemic haloxyfop acid (purity, 98.8%) at a dose of 0, 3, 10 and 30 mg/kg bw per day in corn oil by oral gavage for 7 days. The study did not comply with GLP, as the laboratory was not GLP-certified, but the study was performed in accordance with most of the concepts and principles of GLP. All guinea-pigs were implanted with osmotic pumps containing bromodeoxyuridine (BrdU) at 15 mg/ml on the first day of treatment. After 7 days of treatment, the guinea-pigs were killed by CO_2 asphyxiation and the gall bladders and livers were removed for investigations, including histopathology, activity of acyl CoA oxidase, mean cell replication index (BrdU incorporation) and the number of apoptotic cells (identified by TUNEL staining). There was a dose-dependent increase in body-weight gain during treatment, with the body-weight gain of the group at the highest dose being 358% of control value. There were small dose-related increases in both absolute and relative liver weight, which were statistically significant ($p < 0.05$) at the highest dose only (absolute, 131% of control value; relative, 120%). Acyl CoA oxidase activity was raised (184% of control value) in the group at the highest dose only. No treatment-related liver histopathology was seen at any dose. Some guinea-pigs in the group at 30 mg/kg bw per day showed small increases in cell replication index and apoptotic index, but the group mean effects were not statistically different from the control values. The Meeting concluded that the NOAEL was 10 mg/kg bw per day on the basis of small increases in liver weight and peroxisomal enzyme (acyl CoA oxidase) activity at 30 mg/kg bw per day (Elcombe, 2002d).

Dogs

The 13-week study in dogs given racemic haloxyfop acid (purity, 99.8%), described more fully in section 2.2, incorporated an investigation of effects on hepatic peroxisome proliferation using light microscopy and electron microscopy on samples of liver. Groups of four male and four female beagle dogs had been given oral doses at 0, 2, 5 or 20 mg/kg bw per day. Absolute liver weight, relative to that of controls, was significantly ($p < 0.05$) increased in males at 5 or 20 mg/kg bw per day and in females at 20 mg/kg bw per day; relative liver weight was significantly increased in males and females at 20 mg/kg bw per day. Light microscopy showed slight hepatocellular hypertrophy in males and females at 20 mg/kg bw per day and in females at 5 mg/kg bw per day that was due to increased stores of glycogen (vacuoles stained positive for glycogen but negative for fat). Electron microscopy showed no treatment-related changes. Hepatic activity of cyanide-insensitive beta-oxidation of palmitoyl-CoA (FAOX) significantly increased in males and females at 5 and 20 mg/kg bw per day, but not at 2 mg/kg bw per day. The NOAEL for hepatic effects was 2 mg/kg bw per day on the basis of induction of FAOX at 5 and 20 mg/kg bw per day (Dietz et al., 1987).

In a 12-month study that did not comply with GLP and that is described more fully in section 2.2 (Barna-Lloyd et al., 1984), groups of six male and six female beagle dogs were given diets containing racemic haloxyfop acid (purity, 99.6%) at amounts that provided doses of 0, 0.05, 0.5 or 5 mg/kg bw per day. At the end of the study, livers were weighed and examined by light microscopy, but no adverse effects were seen. Liver samples from three males and three females from each group were prepared for electron microscopy. The resultant micrographs of the centrilobular area at 8000 × magnification were examined for peroxisomal volume density, which was expressed as a percentage of cytoplasmic volume. There were no effects on liver weight, liver histopathology or on peroxisomal volume density. The NOAEL for peroxisome proliferation was the highest dose tested, 5 mg/kg bw per day (Barnard et al., 1986).

Monkeys

In a GLP-compliant 4-week study that is described more fully in section 2.2, groups of two male cynomolgus monkeys were given the sodium salt of racemic haloxyfop acid (purity, 99.6%) in distilled water by nasogastric intubation at dosages of 0, 5 or 20 mg/kg bw per day. An additional two monkeys were given a dose of 20 mg/kg bw per day for 4 weeks, followed by a 4-week recovery period. At the end of the study, livers were weighed and examined by light microscopy and electron microscopy. The electron-micrographs of the centrilobular area at 8000 × magnification were examined for peroxisomal volume density, which was expressed as a percentage of cytoplasmic volume. No effects were seen on liver weight, liver histopathology or on peroxisomal volume density. The morphology of peroxisomes and other organelles in the hepatocytes was unaffected by treatment. The NOAEL for peroxisome proliferation was the highest dose tested, 20 mg/kg bw per day (Gerbig et al., 1985).

In a GLP-compliant 13-week study that is described more fully in section 2.2, groups of four male and four female cynomolgus monkeys were given racemic haloxyfop acid (purity, 99.8%) at a dose of 0, 2, 10 or 30 mg/kg bw per day by nasogastric intubation. The monkeys were killed at the end of the 13 weeks of treatment, autopsied and histopathology was performed on major organs. Livers from the controls and from the group at the highest dose were evaluated by electron microscopy and for enzyme changes associated with peroxisomal fatty acid beta-oxidation (FAOX). The absolute and relative weights of the liver were significantly ($p < 0.05$) increased, relative to those of controls, in males and females of the group at the highest dose. At autopsy, the livers of the females at 10 mg/kg bw per day and males and females at 30 mg/kg bw per day had a pale appearance with an accentuated lobular pattern. Light microscopy showed slight hepatocellular hypertrophy with increased numbers of fatty vacuoles in monkeys at 10 or 30 mg/kg bw per day. Ultrastructural evaluation by electron microscopy of centrilobular hepatocytes showed the livers of monkeys at the highest dose to have a slightly higher cytoplasmic lipid volume density and larger numbers of small fat droplets than controls. Peroxisomal volume densities were similar for the monkeys in the control group and for the group at the highest dose. Hepatic peroxisomal FAOX enzyme activity was not significantly different from that of controls at 30 mg/kg bw per day for males and females. The Meeting concluded that there was no effect on peroxisome proliferation in the livers of cynomolgus monkeys at doses of up to 30 mg/kg bw per day. It was noted, however, that doses of 10 mg/kg bw per day or more caused fatty change in hepatocytes, with a NOAEL of 2 mg/kg bw per day for this effect (Yano et al., 1987).

3. Observations in humans

3.1 Studies in volunteers

Studies of pharmacokinetics in humans given the sodium salt of racemic haloxyfop by oral administration and racemic haloxyfop methyl ester by dermal application are covered in section 1.1, together with the results of a study of people using a formulated product (containing haloxyfop-R methyl ester) as recommended. No adverse health effects were reported in any of the studies (Scortichini et al., 1987).

3.2 Observations in potentially exposed humans

Haloxyfop-R methyl ester had been manufactured by Dow AgroSciences at Drusenheim, France since 1994. There had been no reported incidents of employee exposure since 1995. A review of medical surveillance data for 50 plant employees, including 38 with potential exposure, showed no health effects that could be attributed to exposure to haloxyfop (Dow AgroSciences, 2002).

Between January 1999 and January 2002 in China, there were 82 reported incidents of alleged human health effects associated with an herbicidal product that contained haloxyfop-R methyl ester. Of these incidents, 42 involved oral exposure (22 intentional), 23 involved skin exposure, 5 involved eye exposure and 13 involved inhalation during spraying. In the intentional oral exposures, the signs of toxicity included drowsiness, lethargy, seizures, coma and death. These non-specific signs can be attributed either to the petroleum distillate solvent used as coformulant or to haloxyfop, or both, since they are both contained at high concentrations (about 20% and 10%, respectively) in the commercial preparation. The majority of the non-intentional oral exposures were small and resulted in minor symptoms (Dow AgroSciences, 2002).

Comments

Biochemical aspects

Studies of pharmacokinetics and metabolism were conducted with racemic haloxyfop and haloxyfop-R methyl ester. Oral doses of haloxyfop-R methyl ester or haloxyfop were rapidly and extensively absorbed in all laboratory species tested (mice, rats, dogs and cynomolgus monkeys) and in humans (absorption half-life in men, 0.9 h). Irrespective of whether haloxyfop or haloxyfop-R methyl ester was administered, haloxyfop was the only substance detected in the plasma. *S*-Isomeric forms of haloxyfop underwent rapid and almost complete inversion to *R*-forms in rats, and it was assumed that this also occurred in other species. The highest concentrations of residue were in the liver and kidneys. Biphasic elimination was seen in dogs and cynomolgus monkeys, with a rapid initial phase (half-lives, 1–2 h in dogs and 2.5 h in monkeys) followed by a slow second phase (half-lives, 34 h in dogs and 3 days in monkeys). There was little primary metabolism of haloxyfop in any species tested, but there was some conjugation. Glucuronidation occurred in mice and rats. The major route of elimination was the faeces in mice (recovered radiolabel: males, 79%; and females, 71%), male rats (55–77%) and male dogs (88%), but the urine was the major route of excretion in female rats (68–81%) and cynomolgus monkeys (99%). Men excreted 65–100% of a single oral dose in the urine.

The pharmacokinetics and metabolic data on haloxyfop-R methyl ester and haloxyfop suggest that the results of studies of oral toxicity with racemic haloxyfop methyl ester, haloxyfop or haloxyfop sodium salt should be relevant to the investigation of the toxicity of haloxyfop–R methyl ester and haloxyfop-R, as all stereoisomeric forms and esterified forms end up as the de-esterified *R* enantiomer.

Toxicological data

Haloxyfop administered orally was of moderate acute toxicity in mice, rats and cynomolgus monkeys, with apparently higher toxicity in male rats than in female rats (LD_{50} for haloxyfop-R methyl ester: males, 337 mg/kg bw; females, 545 mg/kg bw). Signs of gastric irritation were seen at high oral doses (1000 mg/kg bw) in one study in rats and signs of hepatotoxicity were seen in another study of acute toxicity in rats. Haloxyfop-R methyl ester was not an irritant to the skin or eyes of rabbits and was not a skin sensitizer in guinea-pigs (Magnusson & Kligman and Buehler tests).

The toxicity of haloxyfop has been investigated in short-term studies in mice, rats, dogs and monkeys. In all these species, haloxyfop caused hepatocellular hypertrophy, which was often associated with increased eosinophilia of the cytoplasm and a more homogeneous appearance to the cytoplasm than in controls. The lowest NOEL for this effect was 0.02 mg/kg bw per day in short-term studies of toxicity in mice and rats. A modest but consistent increase in serum AP activity was seen in mice and rats at 2 mg/kg bw per day, with a NOAEL of 0.2 mg/kg bw per day. Hepatocellular hypertrophy was seen at 5 mg/kg bw per day or greater in a study in dogs, and at 10 mg/kg bw per day or greater in a study in monkeys. In addition to these effects on the liver, thyroid changes were

seen and a NOAEL of 0.2 mg/kg bw per day was identified in the studies in dogs and monkeys. Small decreases in serum cholesterol concentrations and small increases in haematological parameters were measured in dogs receiving haloxyfop at doses below that producing hepatocellular hypertrophy, but were not regarded as toxicologically significant.

Repeated dermal doses of 40 mg/kg bw per day or more of haloxyfop-R methyl ester caused similar effects on the livers of male rats to those seen after repeated oral doses of rats with haloxyfop. A NOAEL was not identified.

Combined long-term studies of toxicity and carcinogenicity were conducted with haloxyfop in mice and rats. In mice, hepatocellular hypertrophy and liver tumours were seen. A dose-related positive trend in the number of animals having one or more liver masses, and statistically significant increases in the incidences of liver tumours (adenoma, and adenoma plus carcinoma in males; carcinoma in females) occurred. Only the increased incidence of liver carcinomas was greater than the historical control range. The LOAEL for production of liver tumours in mice was 0.6 mg/kg bw per day and the NOAEL was 0.065 mg/kg bw per day. Hepatocellular hypertrophy was seen in rats in the absence of an increase in tumour incidence, and the NOAEL was therefore identified as the highest dose tested (0.1 mg/kg bw per day in males, and 1 mg/kg bw per day in females). Haloxyfop was not carcinogenic in rats.

Haloxyfop and haloxyfop-R methyl ester gave negative results in a comprehensive range of studies of genotoxicity in vitro and in vivo. The Meeting concluded that haloxyfop, haloxyfop-R and haloxyfop-R methyl ester are unlikely to be genotoxic.

In order to confirm the hypothesis that the mouse liver tumours observed at 0.6 mg/kg bw per day were the result of a mode of action involving peroxisome proliferation, a series of special studies were performed, including studies in vitro and in vivo in mice, rats, guinea pigs, dogs and cynomolgus monkeys treated orally. It was shown by electron microscopy that exposure to haloxyfop caused proliferation of hepatocellular peroxisomes in mice and rats at 10 mg/kg bw per day, but not in dogs or cynomolgus monkeys at up to 20 mg/kg bw per day. In mice, hepatic CAT was induced at 0.5 mg/kg bw per day or more, and other enzymes were induced at higher doses: catalase, GPD, and FAOX. Furthermore, several genes (ACO, CYP4A10 and CTE-I) that are associated with peroxisome proliferation were upregulated at 0.6 mg/kg bw per day. In rats, CAT, catalase, GPD and FAOX were induced. In guinea-pigs, ACO activity was increased modestly at 30 mg/kg bw per day. In dogs, electron microscopy showed no peroxisome proliferation at doses of up to 20 mg/kg bw per day, but there was increased activity of hepatic FAOX at doses of 5 mg/kg bw per day or more. Doses of up to 30 mg/kg bw per day did not cause hepatocellular peroxisome proliferation in cynomolgus monkeys and FAOX activity in hepatic peroxisomes was unaffected. Studies in vitro showed that peroxisome proliferation did not occur in human hepatocytes exposed to haloxyfop acid or to a positive-control peroxisome proliferator (WY14,643) at doses that caused peroxisome proliferation in hepatocytes from mice.

The negative results of tests for mutagenicity indicate that the tumours in mice were not caused by a genotoxic mechanism. The Meeting used the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans (Boobis et al., 2006) in considering whether the mode of action by which the liver tumours were produced in mice was relevant to humans. It was concluded that the production of liver tumours in mice was by a mode of action that involved hepatocellular peroxisome proliferation and it was noted that this effect was not seen in dogs, cynomolgus monkeys or in cultured human hepatocytes. Hepatocellular peroxisome proliferation has been shown elsewhere to be associated with hepatocellular hyperplasia and liver tumours in rodents, but not in non-rodent species. It has been well established (Klaunig et al., 2003) that chemicals that induce mouse liver tumours via peroxisome proliferator-induced receptor alpha (PPARα) agonism do not pose a risk of hepatocarcinogenesis in humans, because of quantitative dynamic differences in PPARα activation.

The Meeting concluded that haloxyfop is unlikely to pose a carcinogenic risk to humans based on the absence of genotoxicity and the recognition of a mode of action for production of the liver tumours in mice that is not relevant to humans.

Multigeneration studies with haloxyfop in rats showed no effects on reproduction at doses that were not maternally toxic. Low pup body weights (F_{1a}, F_{1b} and F_{2a} pup body weights, 5–10% lower than controls) and low litter weights (6–7% lower in litters of F_{1b} pups on postnatal days 14–21) were reported at 1 mg/kg bw per day in Sprague-Dawley rats in the two-generation study. The NOAEL was 0.065 mg/kg bw per day. In the three-generation study in Fischer 344 rats, the NOAEL was 0.05 mg/kg bw per day on the basis of low pup body weights seen at a dose of 1 mg/kg bw per day in the F_{1b} and F_{2a} pups during weaning and up to termination of the study at age 28 days. The overall NOAEL for the effects on pup body weight was 0.065 mg/kg bw per day.

In a study of developmental toxicity, the fetuses of rats given haloxyfop at a dose of 7.5 mg/kg bw per day showed delayed ossification of thoracic vertebrae. The NOAEL was 1 mg/kg bw per day. Two studies of developmental toxicity were performed in rabbits. In the first, the NOAEL was 7.5 mg/kg bw per day on the basis of an increased incidence of a minor skeletal variation at 20 mg/kg bw per day. Increased maternal mortality, increased number of resorbed implantations and increased number of litters with resorptions were also seen at this dose. In the second study, there was no adverse effect on development, but there was increased maternal mortality at 15 mg/kg bw per day. The NOAEL was 7.5 mg/kg bw per day. The Meeting concluded that haloxyfop is not teratogenic.

No cases of adverse effects were reported in France between 1995 and 2002 among personnel involved in the production and formulation of haloxyfop-based products. A medical review of 50 workers at the plant showed no adverse health findings that could be attributed to exposure to haloxyfop. In China, between January 1999 and January 2002, there were 82 reported incidents of alleged human health effects associated with an herbicidal product that contained haloxyfop-R methyl ester. The majority of the reported incidents concerned small accidental exposures involving minor symptoms. Intentional oral exposures to the commercial preparation were associated with drowsiness, lethargy, seizures, coma and death. These non-specific signs can be attributed either to the petroleum distillate solvent used as coformulant or to haloxyfop, or both, since they are both contained at high concentrations (about 20% and 10%, respectively) in the commercial preparation.

The Meeting noted that there were no qualitative or quantitative differences in the toxicological properties of haloxyfop-R methyl ester and haloxyfop. None of the studies revealed any unexpected effects of haloxyfop-R methyl ester, and the quantitative dose–effect relationships of racemate and *R*-enantiomer were very similar. The data on haloxyfop could therefore be used for the toxicological evaluation of haloxyfop-R methyl ester and haloxyfop-R.

The Meeting concluded that the existing database was adequate to characterize the potential hazard of haloxyfop, haloxyfop-R and their methyl esters to fetuses, infants and children.

Toxicological evaluation

The Meeting concluded that there were sufficient data to bridge the toxicology studies between haloxyfop and haloxyfop-R methyl ester. As the relative molecular mass of haloxyfop methyl ester is very close to that of haloxyfop, there is no need to compensate for differences in relative molecular mass when considering doses.

The Meeting established a group ADI of 0–0.0007 mg/kg bw for racemic haloxyfop, haloxyfop-R and their methyl esters based on a NOAEL of 0.065 mg/kg bw per day for low pup body weight in multigeneration studies in rats, and applying a safety factor of 100.

The Meeting established a group ARfD of 0.08 mg/kg bw for racemic haloxyfop, haloxyfop-R and their methyl esters on the basis of a NOAEL of 7.5 mg/kg bw per day for maternal mortality and increased number of resorptions at higher doses in a study of developmental toxicity in rabbits, and applying a safety factor of 100.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Thirty-six-week study of toxicity[a,e]	Toxicity	0.2 mg/kg bw per day	2 mg/kg bw per day
	Two-year study of carcinogenicity[a,fe]	Toxicity	0.6 mg/kg bw per day[d]	—
		Carcinogenicity	0.065 mg/kg bw per day[d]	0.6 mg/kg bw per day
Rat	Sixteen-week study of toxicity[a,b]	Toxicity	0.2 mg/kg bw per day	2 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a,e]	Toxicity	0.1 mg/kg bw per day	—
		Carcinogenicity	0.1 mg/kg bw per day	—
	Two-generation study of reproductive toxicity[a,e]	Maternal toxicity	1 mg/kg bw per day	—
		Offspring toxicity	0.065 mg/kg bw per day	1 mg/kg bw per day
	Developmental toxicity[b,e]	Maternal toxicity	7.5 mg/kg bw per day	—
		Fetotoxicity	1 mg/kg bw per day	7.5 mg/kg bw per day
Rabbit	Developmental toxicity[b,e]	Maternal toxicity	7.5 mg/kg bw per day	15 mg/kg bw per day
		Embryo- and fetotoxicity	7.5 mg/kg bw per day	15 mg/kg bw per day
Dog	1-year study of toxicity[a,e]	Toxicity	0.5 mg/kg bw per day	5 mg/kg bw per day
Monkey	13-week study of toxicity[c]	Toxicity	2 mg/kg bw per day	10 mg/kg bw per day

[a] Dietary administration with dietary concentrations adjusted weekly to maintain the intended dosages.

[b] Gavage administration.

[c] Nasogastric administration.

[d] Highest dose tested.

[e] Study performed with haloxyfop.

Estimate of acceptable daily intake for humans

0–0.0007 mg/kg bw

Estimate of acute reference dose

0.08 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 haloxyfop (including haloxyfop-R and haloxyfop-R methyl ester)

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and extensive
Distribution	Widely distributed with highest levels in liver and kidneys.
Potential for accumulation	None
Rate and extent of excretion	$T_{½}$ = 6.3 days for clearance from plasma to urine (human). Mostly excreted in urine. Main route of excretion is faeces in mice, male rats and dogs but urine in female rats, cynomolgus monkeys and men.
Metabolism in mammals	Oral haloxyfop methyl ester rapidly transformed to haloxyfop acid on absorption. Little primary metabolism, but some conjugation.
Toxicologically significant compounds in animals, plants and the environment	Haloxyfop
Acute toxicity	
Rat, LD_{50}, oral	300–337 mg/kg bw in males, 545–623 mg/kg bw in females
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rabbit, dermal irritancy	Not irritating
Rabbit, eye irritancy	Not irritating
Guinea-pig, skin sensitization	Not sensitizing (Buehler, and Magnusson & Kligman)
Short-term studies of toxicity	
Target/critical effect	Liver
Lowest relevant oral NOAEL	0.2 mg/kg bw per day (serum alkaline phosphatase activity in mouse)
Lowest relevant dermal NOAEL	< 40 mg/kg bw per day (erythrocytes)
Genotoxicity	
	Not genotoxic in vitro or in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	None
Lowest relevant NOAEL	0.1 mg/kg bw per day (highest dose tested in male rats)
Carcinogenicity	No carcinogenic potential in the rat. Hepatocellular carcinoma in female mice associated with peroxisome proliferation (mode of action not relevant to humans)
Reproductive toxicity	
Reproductive target/critical effects	No reproductive effects Low pup weight
Lowest relevant reproductive NOAEL	Parental: 1 mg/kg bw per day (highest dose tested) Offspring: 0.065 mg/kg bw per day (pup weight)
Developmental target critical effect	Delayed ossification, resorptions
Lowest relevant developmental NOAEL	Maternal: 7.5 mg/kg bw per day (mortality) Developmental: 1 mg/kg bw per day (delayed ossification)
Neurotoxicity/delayed neurotoxicity	
	No specific study; no findings in other studies
Other toxicological studies	
Mechanism studies	Increased hepatocellular peroxisome proliferation in mice, rats and hepatocytes from guinea-pigs No effect on peroxisome proliferation in dogs, monkeys or hepatocytes from humans
Medical data	
	No adverse effects on health of manufacturing workers

Summary for racemic haloxyfop, haloxyfop-R and their methyl esters

	Value	Study	Safety factor
Group ADI	0–0.0007 mg/kg bw per day	Rat, two-generation study of reproductive toxicity	100
Group ARfD	0.08 mg/kg bw	Rabbit, studies of developmental toxicity	100

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PIRIMIPHOS-METHYL (ADDENDUM)

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Explanation

Pirimiphos-methyl is an organophosphorus insecticide and acaricide. Toxicological monographs for pirimiphos-methyl were prepared by the Joint Meeting in 1974, 1976 and 1992. In 1992, an acceptable daily intake (ADI) of 0–0.03 mg/kg bw was established based on a NOAEL of 0.25 mg/kg bw per day in a 28-day and a 58-day study in human volunteers, and a safety factor of 10.

At the request of the Codex Committee on Pesticide Residues (CCPR), the requirement for an acute reference dose (ARfD) was considered on the basis of data from previous evaluations as well as new studies. A number of studies previously evaluated by the JMPR were considered to be possibly relevant for establishing an ARfD and were re-evaluated.

Pirimiphos-methyl was being considered by WHO as a larvicide treatment for drinking-water. For that reason, the WHO programme on guidelines for drinking-water quality had recommended that pirimiphos-methyl be evaluated toxicologically by JMPR.

For pirimiphos-methyl, the specifications were established by the Joint FAO/WHO Meeting on Pesticide Specifications (JMPS) and published as *WHO specifications and evaluations for public health pesticides: pirimiphos-methyl* (2006).[1]

The pivotal studies with pirimiphos-methyl were certified as being compliant with good laboratory practice (GLP). Other available studies were carried out before the Organisation for

[1] Available from: http://www.who.int/whopes/quality/en/Pirimiphos_methyl_eval_may_06.pdf.

Economic Co-operation and Development (OECD) guidelines on GLP were implemented. However, the quality of these studies was considered to be acceptable.

Evaluation for an acute reference dose

1. Biochemical aspects

1.1 Absorption, distribution and excretion

No studies of absorption distribution and excretion were available for the present evaluation. A number of relevant summaries from the 1992 JMPR evaluation are presented below; an asterisk is used to indicate that the text describing these studies was extracted from the JMPR 1992 report.

Rats

Oral administration of 2-^{14}C-ring-labelled pirimiphos-methyl at a dose of 0.6 mg/kg bw to five male rats resulted in a mean urinary excretion of 80.7% and mean faecal excretion of 7.3% in 24 h, indicating rapid absorption. At 96 h, 86.0% and 15.2% of the administered dose had been excreted in urine and faeces, respectively. Nine (unidentified) metabolites were present in the urine (Bratt & Dudley, 1970).

Female rats given 2-^{14}C-pirimiphos-methyl at a dose of 7.5 mg/kg bw orally were bled (cardiac puncture, three rats per time interval) at 0.5, 1, 3, 5, 7 or 24 h after dosing. Maximum blood concentrations (at 0.5 h) were 2–3 µg/ml, declining by 50% 1 h after dosing. By 24 h, concentrations of ^{14}C in blood were 0.2–0.3 µg/ml, and of pirimiphos-methyl, 0.01–0.02 µg/ml. Rats treated for 4 days with 2-^{14}C-pirimiphos-methyl at a dose of 7.5 mg/kg bw per day and sacrificed at intervals of 24 h did not show any increase in blood concentrations with time. Tissue concentrations of total radioactivity in the liver, kidney and fat over the 4 days were generally less than 2 mg pirimiphos-methyl equivalents/kg tissue (concentrations of unchanged pirimiphos-methyl being less than 0.15 mg/kg tissue). There was no evidence of tissue accumulation (Mills, 1976).

Adult male Wistar rats were intubated with ^{14}C-labelled pirimiphos-methyl at a dose of 1 mg/kg bw per day. Four groups of three animals were dosed for 3, 7, 14 or 21 days and sacrificed 24 h after the final dose. A further five groups of three rats were given similar doses for 28 days and sacrificed 1, 3, 7, 14, or 28 days after dosing. For each of the nine groups, one rat that did not receive pirimiphos-methyl was used as a control. After sacrifice, samples of liver, kidney, muscle, fat, erythrocytes and plasma were taken for analyses. Urine and faeces were collected from two rats during the 24 h after the seventh dose. Recovery of ^{14}C from ^{14}C-labelled pirimiphos-methyl added to control tissues was 96.9 ± 5.2%. In all tissue samples taken at all time intervals, the concentration of radioactivity was very low, close to or below detection limits. Concentrations did not increase with repeated dosing. Liver concentrations were fairly constant (0.03 ppm) and similar concentrations were detected in some kidney samples. In other tissues, the concentration of radioactivity was generally below the limits of detection (0.04–0.06 ppm). Three days after cessation of dosing, one animal had detectable concentrations of radioactivity in the kidney. At 7 days and on subsequent days, no residues were found. Excretion was between 70% and 80% of a single dose, after administration of seven consecutive doses, providing evidence for rapid metabolism and elimination rather than poor absorption (Hawkins & Moore, 1979).

Dogs

Groups of one male beagle dog were given capsules containing 2-^{14}C-ring-labelled pirimiphos-methyl at a dose of 18.4 or 16.7 mg/kg bw. Of the administered dose, 64.4% or 82.5% was excreted in the urine, and 17.3% or 13.3% in the faeces, respectively, in 48 h. Nine (unidentified) metabolites were present in the urine (Bratt & Dudley, 1970).

As a thiophosphate, pirimiphos-methyl requires metabolic activation (from P=S to P=O) to inhibit acetylcholinesterase. No data are available on the interindividual variability of P=S oxidation (Tang et al., 2005). Pirimiphos-methyl is highly lipophilic (log K_{ow} = 4.2).

2. Toxicological studies

2.1 Acute toxicity

The acute oral toxicity of pirimiphos-methyl is summarized in Table 1.

Table 1. Results of studies of acute toxicity with pirimiphos-methyl

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	Unknown	Male	Oral	90–94	1180	Clark (1970)
Rat	Unknown	Female	Oral	90–94	2050	Clark (1970)
Rat	Unknown	Male	Oral	90.5	1861	Rajini & Krishnakumari (1988)
		Female	Oral		1667	
Guinea-pig	Unknown	Female	Oral	90–94	1000–2000	Clark (1970)
Rabbit	Unknown	Male	Oral	90–94	1150–2300	Clark (1970)
Cat	Unknown	Female	Oral	90–94	575–1150	Clark (1970)
Dog[a]	Unknown	Male	Oral	90–94	> 1500	Gage (1972)

[a] The study of acute toxicity in dogs, by Gage (1972), was not available for the present evaluation. Data were taken from JMPR 1992.

The clinical signs reported in the studies of acute toxicity, as described by Clark (1970) and Rajini & Krishnakumari (1988), are typical of those resulting from cholinesterase inhibition, i.e. incontinence, salivation, chromolacrimation, tremors, fibrillations, fasciculations and prostration.

2.2 Short-term studies of toxicity

Rats

Groups of 12 male and 12 female rats (age 6–9 weeks) were fed diets containing pirimiphos-methyl (purity, 97%) at 0, 5, 8, 10 or 50 ppm (equivalent to 0, 0.5, 0.8, 1 and 5 mg/kg bw per day) for 28 days. Animals were checked daily for clinical signs. Food consumption (per cage of three rats) and body weight (per individual animal) were measured weekly. Plasma and erythrocyte cholinesterase activity were measured in groups of five males and five females on days −14, −7, 1, 3, 7, 14, 21 and 28. Brain cholinesterase was measured in groups of five males and five females on day 28. At termination on day 28, all animals were examined macroscopically.

There were no treatment-related clinical signs and pirimiphos-methyl had no effect on body-weight gain. Food consumption was slightly reduced (< 6%) in males at 5 ppm (statistically significant) and 8 ppm (not statistically significant). However, since food consumption was not affected in males at 10 and 50 ppm or in any of the groups of females, these findings were not considered to be toxicologically relevant. At termination, gross pathology did not reveal any lesions attributable to pirimiphos-methyl. Inhibition of plasma cholinesterase activity consistently exceeded 20% (up to 62%) in males and females at 50 ppm, at all days of treatment. Sporadic inhibition was noted at 8 and 10 ppm, as was sporadic elevation. However, erythrocyte cholinesterase activity was unaffected by administration of pirimiphos-methyl, even at 50 ppm. At termination, statistically significant inhibition of brain cholinesterase activity was observed at 50 ppm. However, the effects were small (11% in males, 13% in females) and, in the absence of concommitant clinical signs, were not considered to be adverse. Therefore, the no-observed-adverse-effect level (NOAEL) in this study was 50 ppm, equivalent to 5 mg/kg bw per day, i.e. the highest dose tested (Berry & Gore, 1975).

Groups of 30 male Wistar rats (age 8 weeks) were fed diets containing pirimiphos-methyl (purity, 90.5%) at a concentration of 0, 1000 or 1500 ppm (equivalent to 0, 100 and 150 mg/kg bw per day) for 28 days. Necropsy was performed on five rats per group at 7, 14, 21 or 28 days after initiation of exposure, and five rats per treated group were killed at 35 days (i.e. 7 days after withdrawal of pirimiphos-methyl). The fate of the remaining five rats was not reported. At each of the time-points, brain and erythrocyte acetylcholinesterase activity was measured. In addition, activity of plasma pseudocholinesterase and non-specific carboxylesterase in brain liver, plasma and kidney was assessed. Clinical signs and food consumption were recorded daily. Body weight was measured weekly.

It is reported that treatment with pirimiphos-methyl had no effect on clinical signs, food consumption, and body weight (data not shown). Brain and erythrocyte cholinesterase activity showed significant dose-related decreases at all time intervals during exposure (Table 2). Maximum reductions in brain cholinesterase activity were measured from days 14–28. Erythrocyte cholinesterase activity was consistently decreased from days 7–28. Post-exposure recovery of cholinesterase activity occurred in both treatment groups, but brain cholinesterase activity was still biologically significantly depressed (26 and 28% at 1000 ppm and 1500 ppm, respectively) 7 days after cessation of treatment. Plasma cholinesterase activity was variable, but was decreased by 17–44% over the various time intervals, the smallest reduction being at the highest dose. Recovery was complete 7 days after cessation of dosing. Non-specific brain carboxylesterase activity was depressed at 1500 ppm at all time-points, but only after 14 days at 1000 ppm. Recovery was rapid and complete at both doses after withdrawal. Plasma non-specific carboxylesterase activity was markedly depressed at all time-points, but was still significantly depressed after 7 days withdrawal. Renal non-specific carboxylesterase activity was slightly reduced only at 1500 ppm after 14 days treatment and recovered rapidly upon cessation of dosing.

Table 2. Brain and erythrocyte cholinesterase activity (% of control values) in rats given diets containing pirimiphos-methyl

Dietary concentration (ppm)	Cholinesterase	Treatment day				Post-treatment day
		7	14	21	28	7
1000	Brain	78	47	58	55	74
1500		65	39	44	46	72
1000	Erythrocyte	35	32	36	46	92
1500		32	30	25	33	82

From Rajini et al. (1989)

In this study a NOAEL could not be identified. The lowest-observed-adverse-effect level (LOAEL) was 1000 ppm, equivalent to 100 mg/kg bw per day (Rajini et al., 1989).

Five groups of 12 young male rats (strain unknown) were diets containing pirimiphos-methyl (purity, 90.5%) at a concentration of 0, 10, 250, 500 or 1000 ppm (equivalent to 0, 1, 25, 50 and 100 mg/kg bw per day) for 28 days. Animals were checked daily for clinical signs and mortality. Food consumption was measured daily, body weight was measured weekly. At termination blood was sampled by cardiac puncture. Brain, liver, lungs, heart, adrenals, kidneys, spleen and testes were weighed and examined histologically. Brain and plasma cholinesterase and liver and plasma enzyme activity were measured.

There were no effects on mortality, clinical signs, body-weight gain or food intake. Although compound intake was reported to be 0, 4, 100, 200 or 400 mg/rat, it was not indicated over what time-period this was consumed. Therefore, for the present evaluation the intake of compound is based on the standard 10 : 1 ppm to mg/kg bw per day conversion for young rats. A slight increase in liver weight was reported at 1000 ppm. No treatment-related pathological changes were observed in liver, brain, lung, heart, adrenal, kidney, spleen or testes. Increased serum transaminase activity (at 1000 ppm) and increased alkaline phosphatase activity (at 500 and 1000 ppm) were noted. Hepatic transaminases (β-glucuronidase and alkaline phosphatase) were unaffected. Cholinesterase activity (plasma and brain) were dose-dependently inhibited at 250 ppm and above. At 250 and 500 ppm, brain cholinesterase activity was statistically significantly reduced by 18% and 27%, respectively. Based on the 27% reduction in brain cholinesterase activity at 500 ppm, the NOAEL was 250 ppm, equivalent to 25 mg/kg bw per day (Rajini & Krishnakumari, 1988).

Four groups of 25 male and 25 female Alderly Park SPF rats were fed diets containing pirimiphos-methyl (purity, 93.1%) at 0, 8, 80 or 360 ppm (equivalent to 0, 0.4, 4 and 18 mg/kg bw per day) for 90 days. Twenty rats of each sex per group were sacrificed at 90 days; the remaining animals were sacrificed after a 28-day recovery period. The animals were checked daily for clinical signs. Body weight and food consumption were measured weekly, haematology (haemoglobin, erythrocyte volume fraction, total and differential leukocyte counts, reticulocyte counts, mean cell haemoglobin concentration (MCHC), mean corpuscular diameter, platelets, clotting function tests) was performed for five rats of each sex per dose pre-test and after 6 and 13 weeks. Cholinesterase activity in plasma and erythrocytes was tested in five males and five females per group, five times pre-test and after 1, 2, 4, 6, 8, 10 and 12 weeks of dosing and after 1 and 4 weeks during the recovery period. At termination at 90 days, or after the 4-week recovery period, all animals were macroscopically examined, and absolute and relative organ weights (liver, heart, lung, adrenals, kidney, spleen) were assessed for five rats of each sex per group. Histopathology was performed on a selection (19) of organs. Brain cholinesterase activity was assessed in five rats of each sex per dose at 90 days and after the 4-week recovery period. In the report it is not indicated whether the observed effects reached statistical significance.

No treatment-related clinical signs were observed. Compared with controls, body-weight gain in females was reduced by 18% and 21% at 80 ppm and 360 ppm, respectively, but food intake in these groups was slightly increased. No effects were observed on haematological parameters. No effect of treatment on plasma and erythrocyte cholinesterase activity was observed 1 week after the start of dosing. Plasma cholinesterase activity was depressed in males (41–72%) and females (56–88%) during weeks 2–12 at 80 ppm and 360 ppm. Recovery to normal levels of activity was observed 1 week after withdrawal of pirimiphos-methyl. From weeks 2 to 12, erythrocyte cholinesterase activity was depressed in males (39–52%) and females (43–71%) at 360 ppm. In the group at 360 ppm, erythrocyte cholinesterase activity was inhibited by 34% in males and 29% in females 1 week after cessation of treatment. At week 4 of the recovery period, erythrocyte cholinesterase activity was

comparable to control levels. At the end of the treatment period, brain cholinesterase activity was depressed by 20% and 42% in females at 80 and 360 ppm, respectively. At the end of the 4-week recovery period, brain cholinesterase activity was still reduced by 21% and 35% in females at 80 and 360 ppm, respectively. Brain cholinesterase activity in males was not affected by treatment with pirimiphos-methyl. Histopathological examination revealed no treatment-related effects.

Based on the effects on body-weight gain and brain cholinesterase activity in females and on erythrocyte cholinesterase activity in both sexes at 80 ppm, the NOAEL was 8 ppm, equivalent to 0.4 mg/kg bw per day (Clapp & Conning, 1970).

Dogs

Groups of four male and four female beagle dogs were fed gelatin capsules containing pirimiphos-methyl (purity unknown) at a dose of 0, 2, 10 or 25 mg/kg bw per day for 3 months. Animals were checked daily for mortality and clinical signs. Food consumption was recorded twice per day. Body weight was assessed weekly. Ophthalmoscopy was performed before dosing and at weeks 6 and 12. Plasma and erythrocyte cholinesterase activity were assessed five times before dosing, and after 1, 2, 4, 6, 8, 10 and 12 weeks. Haematology, clinical chemistry and urine analysis was performed before dosing and at weeks 6 and 12. Urine analysis and electrocardiography were performed before dosing and at week 12. After 3 months, 2 animals of each sex per group were killed. The remaining animals were allowed to recover for 4 weeks and then killed. All animals were examined macroscopically and a range of organs were weighed and examined histologically. A sample of the left frontal cortex was taken for assessment of cholinesterase activity.

There were no mortalities. In the group at the highest dose, increased incidences of vomiting and loose stools were observed. Furthermore, these animals showed a marked loss of general condition (dry skin, dull coat). In this group, body-weight gain and food consumption were reduced throughout the treatment period. At week 12, a significant reduction in heart rate was found. In the groups at 2 and 10 mg/kg bw per day, body weight gain was similar to that of controls, except for one dog in the group at the intermediate dose, which showed a reduction in body-weight gain from week 6 onwards. Water consumption was not affected. Ophthalmoscopy revealed no effects on the eyes. No consistent effects on haematology, clinical chemistry and urine analysis were observed.

Compared with the values for concurrent controls, erythrocyte cholinesterase activity was consistently and dose-dependently reduced (> 20%) at 10 and 25 mg/kg bw per day, at all time-points after the start of the treatment. Maximum levels of erythrocyte cholinesterase inhibition were reached from weeks 2–4 onwards. Treatment had no effect on brain cholinesterase activity. On the basis of inhibition of erythrocyte cholinesterase activity, the NOAEL was 2 mg/kg bw per day (Noel et al., 1970).

2.3 Reproductive toxicity: developmental toxicity

Rabbits

Groups of 16 female New Zealand White rabbits were given pirimiphos-methyl (purity, 98.8%) at a dose of 0, 12, 24 or 48 mg/kg bw per day (vehicle, corn oil) by gavage once per day during days 6–18 of gestation (day of insemination was denoted day 0). Clinical signs and mortality were examined daily, with particular attention being paid to the 1–2 h after dosing. Body weight and body-weight gain were determined on days 4, 6–19, 22, 26 and 29 of gestation. Food consumption was measured daily. Blood samples were taken from six animals of each group for plasma and erythrocyte cholinesterase analysis on days 5, 19 and 29 of gestation. Time of blood sampling was not specified. At termination on day 29 of gestation, the females were necropsied and ovaries and uterus were examined for number of corpora lutea, implantation sites, live and dead pups and early and late

resorptions. A sample of brain (from the frontal cortex) of approximately 0.1 g was removed from the same six animals in each group and examined for brain cholinesterase activity. Fetuses were weighed, sexed and examined for external, internal and skeletal abnormalities and anomalies. Statements of adherence to quality assurance and GLP were included.

No treatment-related deaths or toxicologically relevant clinical effects were observed. Body weight and food consumption were not significantly affected. The effects of treatment on plasma, erythrocyte and brain cholinesterase activity are described in Table 3.

Table 3. Plasma, erythrocyte and brain cholinesterase activity (% of control values) in rabbits given pirimiphos-methyl by gavage

Time-point	Cholinesterase	Dose (mg/kg bw per day)		
		12	24	48
Day 5	Plasma	102	93	92
	Erythrocyte	98	110	107
Day 19	Plasma	87	63*	50*
	Erythrocyte	79	56*	38*
Day 29	Plasma	102	94	106
	Erythrocyte	110	91	82
	Brain	142	160	62*

From Barton & Hastings (1994).
* $p < 0.05$, statistically significant

At the intermediate and highest doses, maternal toxicity was indicated by depressed erythrocyte cholinesterase activity on day 19. In the group at the highest dose, brain cholinesterase activity was decreased at termination. No toxicologically relevant effects were observed in dams in groups at the lowest dose. On the basis of reduced erythrocyte cholinesterase activity at day 19 in dams at the intermediate dose, the NOAEL for maternal toxicity was 12 mg/kg bw per day.

Pirimiphos-methyl did not induce irreversible structural changes and had no toxicologically relevant effects in fetuses. Therefore the NOAEL for embryo/fetotoxicity was 48 mg/kg bw per day, i.e. the highest dose tested (Barton & Hastings, 1994).

2.4 Special studies: neurotoxicity

Rats

A group of 25 male rats (strain unknown) received a single dose (method of administration not specified) of pirimiphos-methyl (purity, 90.5%) at 1000 mg/kg bw. An additional group of 25 rats served as controls. Five rats per group were sacrificed at 4, 8, 24, 48 or 72 h after dosing and plasma and brain cholinesterase and non-specific carboxylesterase activities were measured. Plasma cholinesterase inhibition was rapid (60% inhibition by 4 h), while inhibition of brain cholinesterase activity was slower (36% by 8 h). Both attained maximum inhibition by 24 h (93% for plasma and 61% for brain). Partial recovery was apparent at 48–72 h for both enzymes, but that for brain was slower. Inhibition of non-specific carboxylesterase activity attained maximum levels (plasma, 80%; and brain, 47%) at 24 h; compared with cholinesterase, inhibition was slightly less, and recovery in plasma was more rapid. In brain, recovery of non-specific carboxylesterase and cholinesterase activity were comparable (Rajini & Krishnakumari, 1988).

Groups of 17 male and 17 female Sprague-Dawley rats received a single oral (gavage) dose of pirimiphos-methyl at 0, 15, 150 or 1500 mg/kg bw in corn oil. In each group, seven animals of each sex were used for neuropathology analysis and ten animals of each sex were allocated for cholinesterase evaluation. Animals were checked daily for viability and clinical signs. Body weight was measured before the test and on days 0, 1, 7, 14 and 15 of treatment. A functional observation battery (FOB) test and a locomotor activity test were performed before the test, at the time of peak effect (± 24 h after dosing—study day 1) and on days 7 and 14 for the seven animals of each sex per group used for neuropathology evaluation and for five animals of each sex per group used for cholinesterase evaluation. In the animals used for cholinesterase evaluation, plasma and erythrocyte cholinesterase activity was determined in five animals of each sex before initiation of dosing, at the time of peak effect (± 24 h after dosing), and on days 7 and 15. Brain cholinesterase activity was assessed in five animals of each sex per group at the time of peak effect (day 1) and at day 15. Blood was collected at euthanization and whole brain weights and regional brain weights were recorded for each animal. In the neuropathology group, all animals were euthanized on day 15 and perfused in situ. A neurohistopathological examination was performed for five animals of each sex in the control group and in the group at 1500 mg/kg bw. Statements of adherence to quality assurance and GLP were included.

No mortality was observed. In animals at 1500 mg/kg bw, clinical signs typical of cholinesterase inhibition were observed, i.e. tremors, lacrimation, staining on body surface, gait alterations, hunched behaviour, exophthalmus, soft stools. These signs were predominantly observed on day 1, although in some animals clinical signs were also observed on days 2–4. In the group at the highest dose, FOB testing on day 1 revealed altered posture (sitting with head lowered, flattened), clonic convulsions (whole body tremors), altered palpebral closure (eyelids drooping or half-closed), lacrimation, salivation, soiled fur, red deposits around eyes, nose and mouth and chromodacryorrhea, catalepsy, altered pupil response and righting reflex, decrease in body temperature, altered hindlimb extensor strength and reduced forelimb and hindlimb grip strength and a reduced rotarod performance. Also, in the group at the highest dose, the motor activity test on day 1 revealed decreased motor activity, gait alterations (walking on tiptoes, hunched body, ataxia), clonic convulsions (whole body tremors; slight or moderate), and decreased arousal and rearing activity. Loss of body weight in the group at the highest dose was observed on the first day of testing. During the next 2 weeks, body weights recovered to control levels. No clinical signs and no effects on FOB or locomotor activity parameters were observed in animals at 15 and 150 mg/kg bw. Treatment with pirimiphos-methyl had no effect on total brain weight, regional brain weight or brain histology in any of the treatment groups.

The effects of treatment with pirimiphos-methyl on cholinesterase activity on day 1 and day 15 are presented in Table 4.

Table 4. Cholinesterase activity (% of control values) in rats given a single oral dose of pirimiphos-methyl by gavage

Effect	Dose (mg/kg bw)					
	15		150		1500	
	Male	Female	Male	Female	Male	Female
Day 1						
Plasma	79*	52*	45*	19*	8*	4*
Erythrocyte	74*	95	61*	79*	29*	37*
Brain sections:						
Hippocampus	97	97	91	86*	35*	42*
Olfactory	91	93	88	94	36*	42*

Midbrain	90*	97	77*	83*	32*	41*
Brainstem	96	98	82*	84*	33*	41*
Cerebellum	93	96	71*	72*	28*	35*
Cortex	100	102	91*	87*	36*	43*
Day 15						
Plasma	119	82	108	101	115	85
Erythrocyte	98	112	88*	97	85*	92
Brain sections:						
Hippocampus	98	91	107	103	76	69
Olfactory	99	90	94	91	79	66*
Midbrain	/3	83	89	78	73*	69*
Brainstem	102	101	95	96	86*	86*
Cerebellum	101	98	105	107	90	91
Cortex	103	100	99	92	71*	70*

From Nemec (1995)
* $p < 0.05$, statistically significant

At day 1, dose-dependent reductions in plasma, erythrocyte and brain cholinesterase activity were observed. At day 15, inhibition of plasma and erythrocyte cholinesterase was < 20% for all treated groups. Regional brain cholinesterase activity was affected in a dose-related manner in the two groups at higher doses. At day 1, some brain regions showed a (significant) inhibition of brain cholinesterase activity of > 20% at the intermediate dose and in all brain regions in the highest dose. At day 15, inhibition of cholinesterase activity of > 20% was observed only in the midbrain of females at the intermediate dose and in most brain regions at the highest dose.

On the basis of inhibition of brain cholinesterase activity in some brain regions at 150 mg/kg bw, the NOAEL was 15 mg/kg bw (Nemec, 1995).

3. Observations in humans

Five healthy men (body weight, 59.5–73 kg bw; age 25 45 years) were given pirimiphos-methyl (purity, 97.8%) at a dose of 0.25 mg/kg bw per day orally for 28 days. Blood samples for measurement of plasma and erythrocyte cholinesterase activity were taken on days −14, −7, 1, 3, 7, 14, 21 and 28.

One subject showed inhibition of plasma cholinesterase activity (21.5%) on day 28. Otherwise changes in cholinesterase activity, both above and below values measured before dosing, were within 12%. Four of five subjects had erythrocyte cholinesterase activity values that were slightly below the pre-exposure values during the last 2 weeks of the study. However, the group means for each time interval did not differ significantly and the variations noted were within the range of variations found by others for normal untreated subjects (Chart et al., 1974).

Three men (body weight, 62–73 kg; age 22–27 years) and four women (body weight, 44–60 kg; age 21–49 years) were given capsules containing pirimiphos-methyl (purity, 97.8%) at a dose of 0.25 mg/kg bw per day for 56 days. Blood samples, for measurement of plasma and erythrocyte cholinesterase activity, liver enzymes and haematology, were taken twice before initiation of dosing, and on days 7, 14, 21, 28, 35, 42, 49 and 56 of the study, and also during the recovery period 7, 14, 21 and 29 days after treatment. Controls comprised two women (body weight, 44 and 46 kg; age 29 and 30 years).

No compound-related effects were observed on liver function (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase or glutanyl transpeptidase activities in plasma), haematology (haemoglobin, erythrocyte volume fraction, MCHC, total and differential leukocyte counts, platelets, erythrocyte sedimentation rate) or erythrocyte cholinesterase activity. Plasma cholinesterase was depressed about 20% in two of four women on days 14, 21 and 28 and in one woman on days 28 and 35. The effect did not increase with time. All values were normal during the withdrawal period (Howard & Gore, 1976).

Comments

Biochemical aspects

No new toxicokinetic studies were available for the present evaluation. The evaluation made by the 1992 JMPR indicated that peak plasma concentrations of radioactivity (after administration of [C^{14}]pirimiphos-methyl) are reached 0.5 h after an oral dose. Pirimiphos-methyl is rapidly excreted. After oral administration of pirimiphos-methyl to male rats, 80.7% and 7.3% of the administered dose was excreted via the urine and faeces, respectively, within 24 h. In dogs, 48 h after dosing at either 18.4 or 16.7 mg/kg bw, urinary excretion was 64.4% or 82.5% and faecal excretion was 17.3% or 13.3%, respectively.

As a thiophosphate, pirimiphos-methyl requires metabolic activation (from P=S to P=O) to inhibit acetylcholinesterase activity. No data were available on the interindividual variability of P=S oxidation. Pirimiphos-methyl is highly lipophilic (log K_{ow} = 4.2).

Toxicological data

The acute oral toxicity of pirimiphos-methyl is low. In the rat, acute oral median lethal dose (LD_{50}) values ranging from 1667 to 2050 mg/kg bw. The clinical signs observed in the LD_{50} experiments are typical of those resulting from inhibition of acetylcholinesterase activity, i.e. incontinence, salivation, chromolacrimation, tremors, fibrillations, fasciculations and prostration.

A number of 28-day and 90-day studies with pirimiphos-methyl were performed in rats and dogs. In all these studies, inhibition of cholinesterase activity was the critical end-point. The overall NOAEL from the studies in rats was 8 ppm, equivalent to 0.4 mg/kg bw per day. The NOAEL in a 90-day study in dogs was 2 mg/kg bw per day. There were no indications that dogs are more sensitive than rats to the effects of pirimiphos-methyl. In these short-term studies it appeared that inhibition of erythrocyte cholinesterase activity reached maximum levels only after 2 weeks of treatment.

In a study of developmental toxicity in rabbits, the NOAEL for maternal toxicity was 12 mg/kg bw per day on the basis of a reduction in erythrocyte acetylcholinesterase activity on day 19 at 24 mg/kg bw per day. No toxicologically relevant effects in fetuses were observed. The NOAEL for embryo-fetotoxicity was 48 mg/kg bw per day. In dams treated at 48 mg/kg bw per day, brain cholinesterase activity was still significantly inhibited at day 29, i.e. 11 days after the last dose.

Two single-dose studies of neurotoxicity in rats were available. In the first, after administration of a high dose (1000 mg/kg bw) of pirimiphos-methyl, maximum inhibition (61%) of brain acetylcholinesterase activity was found after 24 h. Partial recovery was apparent at 48–72 h. In the second single-dose study of neurotoxicity, rats treated with pirimiphos-methyl at 150 or 1500 mg/kg bw showed dose-dependent reductions in erythrocyte and brain acetylcholinesterase activity 24 h after administration. In the animals at the highest dose, brain acetylcholinesterase activity had only partially recovered by day 15 after treatment. On the basis of the inhibition in brain cholinesterase activity at 24 h, the NOAEL was 15 mg/kg bw.

In one 28-day and one 56-day study in humans, pirimiphos-methyl was administered orally at a dose of 0.25 mg/kg bw per day. In neither study was inhibition of erythrocyte acetylcholinesterase activity nor any other toxicologically relevant effect observed.

Toxicological evaluation

The critical effect caused by pirimiphos-methyl is inhibition of acetylcholinesterase activity in the nervous system. Pirimiphos-methyl is not embryo-fetotoxic.

In establishing an ARfD, the Meeting concluded that it is appropriate to use data on inhibition of acetylcholinesterase activity in rats from a single-dose study of neurotoxicity in which a NOAEL of 15 mg/kg bw was identified. Based on this NOAEL, the Meeting established an ARfD of 0.2 mg/kg bw, using a safety factor of 100.

The Meeting considered that it was not appropriate to use a chemical specific adjustment factor, although the occurrence and severity of the adverse effects of acetylcholinesterase inhibitors (directly related to the level of inhibition of cholinesterase activity in the nervous system) are considered to depend on C_{max} rather than the area under the curve. In fact, the Meeting observed that:

- Peak plasma concentrations of radioactivity (after administration of ^{14}C-labelled pirimiphos-methyl) are reached 0.5 h after an oral dose, while maximal inhibition of brain acetylcholinesterase activity appears to occur after about 24 h.
- Pirimiphos-methyl is highly lipophilic ($\log K_{ow} = 4.2$). As a thiophosphate, it requires metabolic activation (from P=S to P=O) to inhibit acetylcholinesterase activity. No data are available on the interindividual variability of P=S oxidation.
- The recovery of brain cholinesterase activity is slow.

Levels relevant for risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity[a]	Neurotoxicity	15 mg/kg bw	150 mg/kg bw
Human	28-day, 56-day toxicity	(Neuro-)toxicity	0.25 mg/kg bw	—[b]

[a] Gavage administration
[b] Highest dose tested

Estimate of acute reference dose

0.2 mg/kg bw

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

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

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QUINOXYFEN

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Explanation

Quinoxyfen is the International Organization of Standardization (ISO) approved name for 5,7-dichloro-4-(4-fluorophenoxy) quinoline, a halogenated quinoline fungicide that acts against powdery mildew in cereal crops. The proposed fungicidal mechanism of action is disruption of signal transduction by targeting of G-proteins.

Quinoxyfen has not been evaluated previously by the JMPR and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide residues (CCPR).

In dietary studies with quinoxyfen, variable levels of incorporation were used to achieve an approximately constant level of exposure throughout the study. All the pivotal studies contained certificates of compliance with good laboratory practice (GLP).

The 2005 CCPR selected quinoxyfen as the second compound for a FAO/WHO/OECD pilot project on work-sharing (see Annex 1, reference *107*). The text of the working paper was based extensively on existing documents prepared by regulatory authorities in Australia, the European Union and the United States of America (USA).

Evaluation for acceptable daily intake

Toxicity and toxicokinetic studies with quinoxyfen have been performed over a period of approximately 15 years starting in 1987. Most studies contained documentation stating compliance with the principles of GLP and the GLP status of the individual studies is identified in the summary text. All studies were considered to have complied with the basic requirements of the applicable Organisation for Economic Co-operation and Development (OECD) or equivalent national test guidelines, unless a comment to the contrary is given in the summary text.

Quinoxyfen is also known by the development codes DE-795 or XDE-795.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

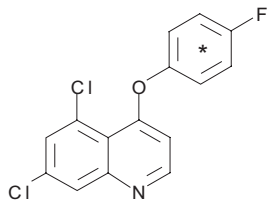
(a) Oral administration

Rats

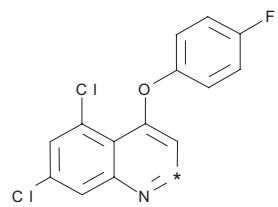
The absorption, distribution, excretion and metabolism of quinoxyfen were investigated in Fisher 344 rats. The rats were aged 7–8 weeks and body weights were 180–250 g in males and 130–180 g in females. A series of tests were conducted using uniformly phenyl ring-labelled ^{14}C-quinoxyfen (radiochemical purity, 98.5%) or quinoline ring-labelled (radiochemical purity, > 99%) ^{14}C-quinoxyfen (Figure 1). The radiolabelled quinoxyfen was suspended in 0.5% aqueous methylcellulose and administered by gavage at a dose of 10 or 500 mg/kg bw to groups of male and female rats, including bile-duct cannulated animals. The doses were based on the findings of the 13-week dietary study in rats (Szabo et al., 1992). The stability and concentrations of quinoxyfen in the dosing solutions were confirmed analytically. Radioactivity in urine, faeces, cage wash, blood, plasma, organs and tissues were measured using liquid scintillation counting (LSC) after appropriate preparatory treatment. Metabolites in the urine, faeces, bile and blood were quantified, characterized and identified using high-performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS) techniques. Reference standards were used as appropriate for the identification of metabolites. Enzymatic incubation techniques (using β-glucuronidase and sulfatase) and acid hydrolysis were used to separate and identify metabolites. Only a limited number of metabolites were identified.

Figure 1. Chemical structure of quinoxyfen and position of radiolabels

^{14}C-Phenyl-labelled quinoxyfen



^{14}C-Quinoline-labelled quinoxyfen



* Position of radiolabel.

In a preliminary study that complied with GLP, two groups of Fisher F344 rats (one male and one female) were administered by gavage a target dose of 10 mg/kg bw of either 2-^{14}C-quinoline ring-labelled or uniformly labelled phenyl ring-labelled ^{14}C-quinoxyfen. Urine, faeces, and expired air were collected at several time-points and analysed for radioactivity. At 72 h after dosing, the rats were killed and the radioactivity in tissues and remaining carcass was quantified.

The distribution of radiolabel in excreta and in tissues and carcass is shown in Table 1. The total amount of radiolabel recovered by 72 h after administration of phenyl ring-labelled or quinoline ring-labelled ^{14}C-quinoxyfen was approximately 80-90% of the total administered radiolabel. For the phenyl ring-labelled treatment, renal excretion of radiolabel (45–49%) was greater than excretion in faeces (38–40%). However, quinoline ring-labelled ^{14}C-quinoxyfen was excreted predominantly in faeces (66–68%) and renal excretion (15%) was the minor route of excretion. The amount of radiolabel expired was minimal (0.4–0.6%) for phenyl and quinoline ring-labelled ^{14}C-quinoxyfen. Retention of radiolabel in tissues/carcass was low (≤ 2%) for phenyl- and quinoline-labelled ^{14}C-quinoxyfen. The findings suggest a rapid and almost complete absorption, distribution and metabolism of quinoxyfen. There were no apparent sex differences in the pattern of elimination of radiolabel. The differences in the distribution of radiolabel in the faeces between phenyl and quinoline-labelled quinoxyfen and urine suggests the separation of the phenyl and quinoline rings and that the metabolites produced have significantly different molecular weights and properties, hence the different routes of elimination. (Schumann et al., 1995)

Table 1. Balance of radioactivity and excretion patterns in rats 72 h after dosing with ^{14}C-phenyl- or ^{14}C-quinoline-labelled quinoxyfen

Sample	Radioactivity (% of administered dose)			
	Phenyl ring-labelled quinoxyfen at 10 mg/kg bw		Quinoline ring-labelled quinoxyfen at 10 mg/kg bw	
	Male	Female	Male	Female
Urine	48.68	44.91	15.09	14.93
Faeces	38.16	39.75	65.76	67.98
Final cage wash	0.45	1.79	0.48	0.19
Expired $^{14}CO_2$	0.60	0.55	0.51	0.35
Tissues/carcass	2.08	1.04	1.65	1.30
Total	89.97	88.04	83.50	84.76

From Schumann et al. (1995)

In the main study, which complied with GLP, the balance and excretion patterns of 2-quinoline ring-labelled quinoxyfen and the amount of residual radioactivity in blood, organs and tissues were determined after the following dosing regimen:

— a single oral dose at 10 mg/kg bw after jugular-vein cannulation;

— a single oral dose at 500 mg/kg bw after jugular-vein cannulation;

— 14 repeated daily oral doses of unlabelled quinoxyfen at 10 mg/kg bw followed by a single dose of radiolabelled quinoxyfen at at 10 mg/kg bw, after jugular-vein cannulation;

— a single oral dose at 10 mg/kg bw after bile-duct cannulation; or

— a single oral dose at 500 mg/kg bw after bile-duct cannulation.

The jugular-vein cannulated rats (five males and five females per group) were killed at 48 h after dosing. The bile-duct cannulated rats (three males per group) were killed at 24 h after dosing. Blood samples were taken from the jugular cannulae at 0.25, 0.5, 0.75, 1, 1.5, 3, 6, 12, 24 and 48 h. Urine, faeces and bile were collected regularly throughout the study.

Table 2 Balance of radioactivity and excretion patterns in rats 48 h after dosing with ^{14}C-quinoline-labelled quinoxyfen

Sample	Radioactivity (% of administered dose)					
	Single dose (mg/kg bw)				Repeated doses[a] (mg/kg bw)	
	10		500		10	10
	Male	Female	Male	Female	Male	Female
Urine	14.76	16.95	13.91	13.40	15.85	19.71
Faeces	78.28	71.53	67.97	69.64	74.19	72.43
Final cage wash	0.58	0.55	0.26	0.79	1.20	0.64
Expired $^{14}CO_2$	NA	NA	0.25	0.24	NA	NA
Tissues/carcass	1.82	2.63	5.45	7.17	1.33	1.70
Contents of gastrointestinal tract	0.72	2.74	1.97	2.02	0.79	0.94
Total	96.16	94.41	89.81	93.27	93.37	95.42

From Schumann et al. (1995)

NA, not analysed.

[a] Fourteen doses of unlabelled quinoxyfen followed by a single dose of radiolabelled quinoxyfen.

Peak plasma radioactivity (C_{max}) was detected at approximately 0.5 h (2–3 µg equiv./g) at 10 mg/kg bw and approximately 1.5 h (80–90 mg equiv./g) at 500 mg/kg bw. The elimination of plasma radioactivity followed a biphasic pattern with half-lives (t½) for the rapid and slow phases for the dose at 10 mg/kg bw of < 1 h and 15–19 h respectively, and 2–3 h and 18–22 h for the dose at 500 mg/kg bw. There were no significant differences between the repeat-dose and single-dose groups. The elimination of radioactivity from blood followed a similar pattern. The area-under-the-curve (AUC) of the plasma versus time graph for the elimination of radiolabel in plasma (0–48 h) was 22.3, 27.3 and 922 µg equiv. h/g in males and 30.4, 29.6 and 963 µg equiv. h/g in females after treatments with a single radiolabelled dose, a single radiolabelled dose after repeated dosing and 500 mg/kg bw ^{14}C-quinoxyfen respectively.

By 24 h after dosing, 68–85% of the administered radioactivity had been recovered in the faeces and urine, indicating rapid elimination from the body. After 48 h, 90-96% of the administered ^{14}C-quinoline ring-labelled quinoxyfen was recovered in the urine, faeces, cage wash and tissues (Table 2). The faeces represented the major route of elimination as 68–78% of the administered dose was eliminated via this route in 48 h, while 13–20% was eliminated in the urine. Urinary half-lives

ranged from 6 to 10 h. The tissues and carcass accounted for 1–7%, contents of the gastrointestinal tract for < 3% and final cage wash for < 1% of the administered dose. There were no sex differences in the distribution of radiolabel. Repeated administration of quinoxyfen did not affect the distribution of the radiolabelled dose.

Comparison of the relative concentrations in bile and urine of intact and bile-duct cannulated rats (Tables 2 & 3) indicated that enterohepatic recirculation is extensive with quinoxyfen at a dose of 10 mg/kg bw. In bileduct cannulated rats, there was a marked difference in the amount of radiolabel in the faeces of rats at 10 mg/kg bw (14.3%) compared with those at 500 mg/kg bw (57.3%) and in the amount of radiolabel in the bile of rats at 10 mg/kg bw (54.4%) compared with those at 500 mg/kg bw (21.4%). These findings indicated that absorption of quinoxyfen at a dose of 500 mg/kg bw dose was saturated.

Table 3. Distribution of radiolabel in bile-duct cannulated male rats 24 h after dosing with ^{14}C-quinoline-labelled quinoxyfen

Sample	Radioactivity (% of administered dose)	
	10 mg/kg bw	500 mg/kg bw
Urine	10.5	2.73
Faeces	14.33	57.30
Final cage wash	1.31	0.60
Carcass	5.16	2.16
Contents of the gastrointestinal tract	1.80	9.73
Skin	0.38	0.18
Bile	54.35	21.44
Total	87.85	94.14

From Schumann et al. (1995)

The distribution of radiolabel in organs and tissues (percentage of administered dose/g tissue) 48 h after treatment with a single dose of quinoline ring-labelled quinoxyfen at 10 mg/kg bw showed that the highest amounts of radiolabel were present in perirenal fat (0.12 in males and 0.35 females) > ovaries (0.07) > liver (0.027 in males and 0.045 in females) and kidneys (0.014 in males and 0.033 in females). Significant levels were found in the skin (Table 3). Similar amounts were obtained after repeated dosing and a similar pattern after the higher dose of 500 mg/kg bw. There were no data on tissue levels at times approximating to the plasma C_{max} (Schumann et al., 1995)

Goats

The metabolism, distribution, and elimination of ^{14}C-quinoxyfen, labelled either in the phenoxy ring or the quinoline ring, was investigated in lactating dairy goats (n = 5; 51–60 kg bw). Two goats were orally dosed with phenoxy ^{14}C-quinoxyfen (purity, > 98%), twice per day for five consecutive days, at a concentration of 10.7 mg/kg feed. Similarly, two goats were treated with quinoline ^{14}C-quinoxyfen, twice per day for five consecutive days, at a concentration of 11.7 mg/kg feed. The remaining goat was used as the untreated control animal. Urine and faeces were collected at intervals of 24 h until sacrifice. Milk samples were collected before the first treatment with quinoxyfen, and then twice daily throughout the study period until animals were sacrificed. The weights of urine, faeces, cage wash and milk samples were recorded, and total radioactivity was measured using LSC. Faecal samples were homogenized with water and subjected to combustion analysis before analysis by LSC. Goats were sacrificed 16 h after the final dose, and samples of the following fluids/tissues were collected for total radiolabelled residue (TRR) analysis: whole blood, plasma, liver, kidney, skeletal muscle, subcutaneous fat, omental fat, perirenal fat, gastrointestinal tract and contents, and carcass. Tissue samples were analysed for their TRR content using combustion analysis and LSC.

Concentrations of total radioactivity in tissues from individual goats for the phenoxy label were: liver, 1.03 and 0.93 mg/kg; kidney, 0.29 and 0.34 mg/kg; muscle, 0.022 and 0.032 mg/kg; perirenal fat, 0.18 and 0.19 mg/kg; omental fat, 0.20 and 0.17 mg/kg; and subcutaneous fat, 0.12 and 0.12 mg/kg. Concentrations of total radioactivity in tissues for the quinoline label were: liver, 0.94 and 1.5 mg/kg; kidney, 0.22 and and 0.17 mg/kg; muscle, 0.015 and 0.032 mg/kg; perirenal fat, 0.13 and 0.32 mg/kg; omental fat, 0.12 and 0.26 mg/kg; and subcutaneous fat, 0.073 and 0.19 mg/kg. Concentrations of radioactivity in milk at 16 h after the final dose were 0.074 and 0.11 mg/kg for the phenoxy label and 0.049 and 0.064 mg/kg for the quinoline label (Dunsire & Paul, 1995).

(b) Dermal administration

No data were available.

(c) Exposure by inhalation

No data were available.

1.2 Metabolism

A proposed metabolic pathway for quinoxyfen is given in Figure 2.

HPLC separation of pooled 0–12 h urine samples from the preliminary study in rats given phenyl-ring-labelled ^{14}C-quinoxyfen produced eight peaks that were designated P_1–P7 and P10. Peak P5 was the major urinary fraction in the unhydrolysed urine sample (80% and 77.4% in the male and female respectively) followed by P3 (7.9% and 9.5%), P_1 (4.2% and 4.7%) and P6 (3.1% and 3.0%). The remaining peaks contained less than 3% of the urinary radiolabel.

Acid hydrolysis of the urine produced a significant change in the HPLC profile. The major fraction in unhydrolysed urine was reduced to only 2.4% of the urine fraction; instead, P8 (not detectable in the unhydrolysed sample) was the major fraction (73.6%). This suggested that P5 might be a conjugate of P8. The P8 fraction was found to co-elute with the standard for 4-fluorophenol. The remaining minor peaks after acid hydrolysis were P3 (11.7%), P9 (7.1%) and P_1 (5.2%), but these metabolites were not identified. The standards used were 4-fluorophenol, 2-hydroxy-quinoxyfen and parent quinoxyfen. Parent quinoxyfen and 2-hydroxy-quinoxyfen retention times did not correspond to that of any of the HPLC urinary fractions. Faecal metabolites from phenyl-^{14}C quinoxyfen showed a similar pattern to those from quinoline-^{14}C quinoxyfen

No further data were provided on the metabolism of the quinoline ring-labelled ^{14}C-quinoxyfen treated rats from the preliminary study (Schumann et al., 1995).

Analysis of blood samples showed that quinoxyfen was rapidly metabolized in rats, with the AUC for total radioactivity being approximately 30 times that for the quinoxyfen.

HPLC separation of pooled urine samples from groups receiving quinoline-^{14}C quinoxyfen in the main study before and after acid hydrolysis produced up to 16 radiolabelled peaks that were designated Q1–Q16. In male rats receiving a single dose at 10 mg/kg bw, eight peaks were identified in unhydrolysed urine: Q3, Q4, Q7, Q8, Q9, Q11, Q12 and Q13. In females rats receiving a single dose at 10 mg/kg bw, four additional peaks were detected: Q2, Q5, Q10 and Q15 (repeated-dose only). For the males and females at 500 mg/kg bw, the peaks in the radiochromatogram of pooled 0–12 h urine samples were Q7, Q8, Q9, Q10 (female only), Q11, Q12 and Q13. The major peaks were Q11 (13–33%), Q8 (13–24%), Q9 (9–24%), Q12 (10–18%), Q13 (6–15%), Q7 (4–11%) and Q4 (< 13%). The only clearly identified urinary metabolite was 5,7 dichloro-4-hydroxyquinoline (DCHQ), which was found to co-elute with peak Q13. Acid hydrolysis resulted in a two- to four-times increase in Q13 (24–65%). In the males rats receiving a single dose at 10 mg/kg bw, increases were observed in

peaks Q3, Q6 and Q14, while peaks Q1 and Q2 were increased after acid hydrolysis. In rats receiving a single dose at 500 mg/kg bw, peak Q14 was increased in males. Concomitantly, peaks Q4, Q5, Q7, Q8 and Q10 were observed to disappear from the respective radiochromatograms, while Q3 and Q9 were markedly reduced. Enzyme hydrolysis did not affect the metabolite profile.

HPLC separation of bile samples (taken at various time-points, 0–24 h) from male rats receiving quinoline ring-labelled quinoxyfen at 10 mg/kg bw or 500 mg/kg bw produced six peaks (B2–B7). Peaks B6 and B7 constituted 20–66% and 26–59% of biliary excretion at various time-points. For the 10 mg/kg bw rats, the amount of radiolabel in the peaks at various time-points were B2 6–15%; B5 4–15%; B3 and B4 < 5%. Only peaks B6 and B7 were detected in the samples from rats at 500 mg/kg bw, but this was attributed to the higher detection limits required by the investigating laboratory. Additional peaks B1, B8, B9 and B10 were detected after enzyme hydrolysis. Peak B7 was noted to disappear with the appearance of peak B10 (approximately 25%) suggesting that B7 is the glucuronide or sulfatase of B10. Acid hydrolysis of the bile samples from rats at 10 or 500 mg/kg bw resulted in an increase in B6 by 20% of the biliary radioactivity compared with the control. Peaks B1, B8 and B9 appeared in the sample from rats at 10 mg/kg bw, but were not detected at 500 mg/kg bw. B10 eluted with quinoxyfen, but MS showed that the key ions were 16 mass units greater than the corresponding fragment ions from quinoxyfen. It was concluded that two metabolites associated with peak B10 were isomers of fluorophenyl-ring hydroxylated quinoxyfen.

Faecal samples exhibited incomplete extraction, typically < 65%. At 500 mg/kg bw, the main faecal metabolite was F8, which co-eluted with quinoxyfen. The primary faecal metabolite at 10 mg/kg bw was F6 which was shown to consist of two hydroxylated isomers, equivalent to the bile metabolite B10. The pattern of metabolites in faeces was essentially independent of radiolabel position (Schumann et al., 1995)

In a GLP-compliant study conducted in 2001, a group of four male Fischer F344 rats received quinoxyfen (purity, 97.4%; lot DECO-97-152-1) at a dose of 500 mg/kg bw by gavage in 0.5% methylcellulose. Excreta were collected at 24-h intervals for 48 h after dosing, when the animals were sacrificed. The primary aim was to investigate hydroxylation at the 3- and 6- positions of the quinoline ring of quinoxyfen. Excreta samples were pooled, treated with sulfatase/glucuronidase, extracted and analysed using electrospray ionization LC/MS and LC/MS/MS to determine levels of 3-hydroxy and 6-hydroxy-quinoxyfen.

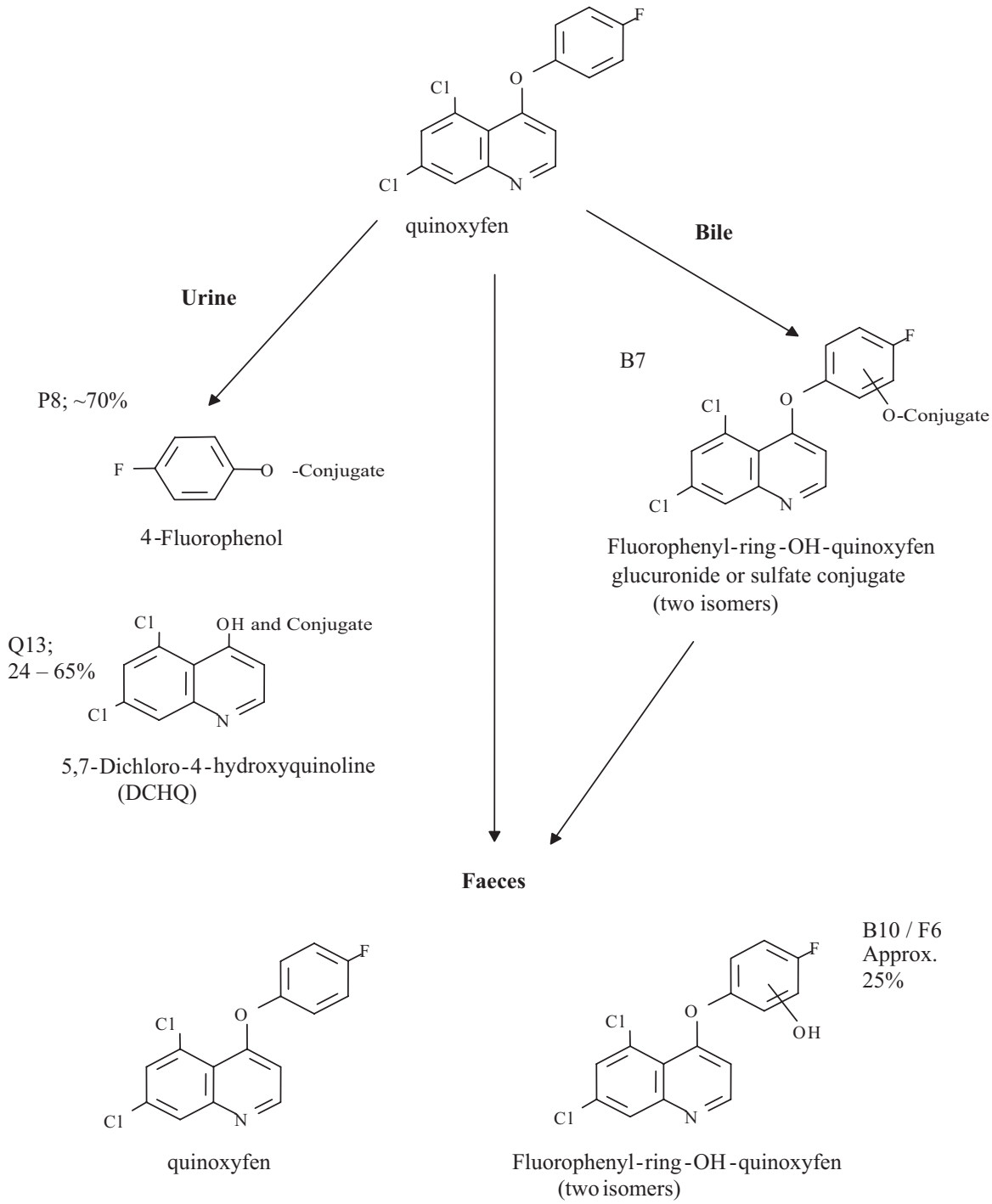
Only limited data were presented in the report owing to the very low levels of hydroxy-metabolites detected. In the 0–24 h faecal sample, 0.012% of the administered dose was present as 3-hydroxy-quinoxyfen, primarily as a conjugate. Initially, low levels of 3-hydroxy-quinoxyfen were detected in urine samples, but this was not confirmed in subsequent analyses. Further analytical work by Pearson & Reeves (2005) indicated that the compound thought to be 3-hydroxy-quinoxyfen was actually 2-oxo-quinoxyfen. No detectable levels of 6-hydroxy-quinoxyfen were found in the urine or faeces, representing < 0.0004% and < 0.004% respectively of the administered dose (Brzak, 2001).

Goats

TRR in excreta (urine, faeces), milk and tissues (kidney, liver, fat) from goats (see Dunsire & Paul, 1995, above) were characterized and/or identified by TLC and HPLC or GC/MS. The major component identified in liver was a conjugated form of quinoxyfen (approximately 10–15% TRR). The only components identified in kidney were quinoxyfen (approximately 3% TRR) and 5,7-dichlorohydroxyquinoline (DCHQ; approximately 2% TRR), with no apparent quinoxyfen conjugates present. In both kidney and liver, major portions of the TRR were characterized as polar based on TLC and HPLC characteristics. The major component present in fat was quinoxyfen (approximately 90% TRR), while milk contained quinoxyfen (approximately 40% TRR) and some very polar material. Small amounts of radioactivity corresponding to 4-fluorophenol, DCHQ, and

several hydroxy-quinoxyfen metabolites were also present in the liver. Small amount of radioactivity corresponding to 2-oxo quinoxyfen, DCHQ, and isomeric hydroxy quinoxyfens were found in the milk. For both labels, hydroxy metabolites and parent compound were the major components present in faeces, while the urine contained mainly a polar component that was easily hydrolysed to 4-fluorophenol or DCHQ (Dunsire & Paul, 1995).

Figure 2. Proposed metabolic pathway for quinoxyfen in rats



2. Toxicological studies

2.1 Acute toxicity

(a) General toxicity

In a series of GLP-compliant studies, quinoxyfen was shown to be of low acute toxicity in rats and rabbits exposed orally, dermally or by inhalation (Table 4). The only signs of toxicity at the limit test doses in the studies of exposure orally or by inhalation were urinary and/or faecal staining in the perineal area, transient reductions in body weight or decreased activity. No clinical signs were reported in the study of dermal toxicity.

Table 4. Results of studies of acute toxicity with quinoxyfen

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Purity (%)	Vehicle	Reference
Rat	F344	M & F	Oral	> 5000	—	97.4	Corn oil	Gilbert (1994a)
Rabbit	NZW	M & F	Dermal (4-h)	> 2000	—	97.4	None	Gilbert (1994b)
Rat	Wistar	M & F	Inhalation (4-h, nose-only)	—	> 3.38(MMAD, 3.6 μm)	97.4	None	Beekman (1994)

F, female; M, male; MMAD, mass median aerodynamic diameter; NZW; New Zealand White.

(b) Ocular irritation, dermal irritation and dermal sensitization

Quinoxyfen was not irritating to skin (Gilbert, 1994c), but was slightly irritating to rabbits' eyes (Gilbert, 1994d). A study of skin sensitization by the Buehler method gave negative results (Gilbert, 1994e), but a second study using the Magnusson & Kligman maximization method produced clear evidence of skin sensitization (Johnson, 1995).

2.2 Short-term studies of toxicity

Oral toxicity with quinoxyfen was investigated in 28-day dietary studies in rats and dogs, 90-day dietary studies in mice, rats and dogs; and in a 52-week dietary study in dogs. Dermal toxicity after repeat doses was investigated in a 28-day study in rats. No studies with repeated doses administered by inhalation had been performed.

(a) Oral administration

Mice

In a GLP-compliant study, groups of 10 male and 10 female CD-1 mice were given diets containing quinoxyfen (purity, 98.7%) at variable concentrations to give nominal doses of 0 (control), 10, 50, 100 or 500 mg/kg bw per day for 13 weeks. The mice were observed for in-life effects and parameters including haematology, clinical chemistry, organ weights, gross and microscopic examination of organs and tissues. Histopathology of all organs and tissues was performed for the control group and for the group at 500 mg/kg bw per day, but the liver with gall bladder, kidneys, lungs and gross lesions only were examined for the groups at lower doses. At the start of treatment, the body weights were 17.3–30.4 g in male mice and 19.5–24.3 g in female mice. The daily intake of quinoxyfen was calculated from weekly assessments of feed consumption. The achieved mean daily intakes were 10, 50, 101 and 507 mg/kg bw per day in males and 10, 52, 105 and 523 mg/kg bw per day in females.

There were no mortalities or adverse effects on the general health, condition or behaviour of the animals during the study. No statistically significant treatment-related differences in feed consumption were observed compared to controls. Although not statistically significant, the mean body weight of males at 500 mg/kg bw per day was slightly lower than that of controls throughout the study (Table 5). However, body weights in females were not affected.

There were no consistent, significant treatment-related differences in clinical chemistry and haematology parameters between treated and control animals. Gross examination at necropsy did not reveal any treatment-related findings. Terminal fasted body weights did not show any significant intergroup differences. There was a significant increase in the relative and absolute liver weight at the 500 mg/kg bw per day. Histopathology revealed slight or moderate centrilobular and midzonal hepatocellular hypertrophy at 500 mg/kg bw per day only. Very slight individual cell hepatocellular necrosis was observed in 3 out of 10 males and 4 out of 10 females at the highest dose (Table 5).

The no-observed-adverse-effect level (NOAEL) was 101 mg/kg bw per day on the basis of liver weight increases, hepatocellular hypertrophy, and histopathological changes (including slight necrosis) in the liver at 500 mg/kg bw per day (Grandjean & Szabo, 1992).

Table 5. Findings in mice receiving diets containing quinoxyfen for 90 days

Finding[a]	Nominal dose (mg/kg bw per day)				
	0	10	50	100	500
Males					
Total leukocyte count ($\times 10^3$)	6.9 ± 2.8	7.4 ± 1.7	7.7 ± 1.2	7.9 ± 3.8	9.4 ± 9.0
Albumin (g/dl)	2.9	2.7	2.6	2.7	2.8
Terminal body weight (g)	37	33	35	36	34
Liver weight, absolute (g)	1.9	1.7	1.7	1.9	2.3
Liver weight, relative (% bw)	5.2	5.0	4.8	5.3	6.7*
Hepatocellular hypertrophy (moderate) (*n*)	0	0	0	0	10*
Hepatocellular necrosis (*n*)	0	0	0	0	3
Females					
Total leukocyte count ($\times 10^3$)	4.9 ± 2.0	5.6 ± 1.7	5.1 ± 1.7	4.3 ± 1.7	5.4 ± 2.4
Albumin (g/dl)	3.0	2.8	2.7	2.7	2.7
Body weight (g)	29	30	29	29	30
Liver weight, absolute (g)	1.3	1.6	1.6	1.6	2.1*
Liver weight, relative (% bw)	5.4	5.4	5.7	5.5	7.0*
Hepatocellular hypertrophy (moderate) (*n*)	0	0	0	0	8*
Hepatocellular necrosis (*n*)	0	0	0	0	4

From Grandjean & Szabo (1992)

[a] n = 10 males and 10 females per group.

* $p < 0.05$

Rats

In a GLP-compliant study, groups of five male and five female Fischer 344 rats were given diets containing quinoxyfen (purity, 97.6%) at variable concentrations, to give target doses of 0 (controls), 250, 500 or 1000 mg/kg bw per day for 4 weeks. The rats were observed for in-life effects and parameters including haematology, clinical chemistry, urine analysis, organ weights, gross and microscopic examinations of organs and tissues. The histopathological examinations were limited to the liver, kidneys and testes. At the start of treatment, the body weight of male rats was 113.2–129.5 g and that of female rats was 105.7–121.4 g. The achieved compound intakes were 272, 549 and 1061 mg/kg bw per day in males and 267, 531 and 977 mg/kg bw per day in females.

No mortalities occurred during the study period. There were no overt signs of toxicity. A dose-related reduction in food consumption was observed at all doses when compared with controls. Although the lower feed consumption in rats treated with quinoxyfen was reported by the investigating laboratory to be indicative of the unpalatability of the diet, it is noted that the target doses were achieved. Body-weight gains were statistically significantly reduced at all doses. The terminal body weights were 89%, 83% and 67% of the control values in males and 93%, 89% and 84% of the control values in females at doses of 250, 500 and 1000 mg/kg bw per day, respectively. Clinical chemistry, urine analysis and haematology investigations did not reveal any significant treatment-related changes; only a reduction in plasma concentrations of glucose occurred. Statistically significant changes in organ weights were considered to be probably related to the differences in body weight and, in the absence of any histopathological changes, of limited toxicological significance. Gross examination at necropsy revealed small bilaterally atrophic testes in rats in the group at 1000 mg/kg bw per day (three out of five male rats). Histopathology revealed a moderate to severe decrease in spermatogenesis of the seminiferous epithelium in the testes of four out of five rats. The changes in the morphology of the testes that was associated with reduced testicular weight were noted by the study investigator to be probably related to the body weight changes. These findings in the testes were notably not observed in subsequent studies in rats given lower doses.

A NOAEL could not be determined due to reductions in food consumption and body-weight gain (7–11%) at the lowest dose tested, 250 mg/kg bw per day (Szabo & Davis, 1992).

In a GLP compliant study, groups of 10 male and 10 female Fischer 344 rats were given diets containing quinoxyfen (purity, 98.7%) at a dose of 0 (control), 10, 100 or 250 mg/kg bw per day for 13 weeks. All animals were killed at the end of the treatment period and underwent necropsy. Additional satellite groups of 10 male and 10 female rats were fed doses of 0 or 250 mg/kg bw per day for 13 weeks and then kept on a quinoxyfen-free diet for an additional 4 weeks in order to investigate recovery from any effects. The achieved daily intakes of quinoxyfen were 10, 102 and 253 mg/kg bw per day) in males and 10, 100 and 249 mg/kg bw per day in females at nominal doses of 10, 100 and 250 mg/kg bw per day, respectively. The parameters investigated included in-life observations, a battery of functional tests, haematology, clinical chemistry, urine analysis, organ weights, gross and histopathological examinations of organs and tissues. Histopathology was limited to the adrenal glands, kidneys, liver, lungs, testes and gross lesions for the groups at 10 and 100 mg/kg bw per day. At the start of the study, the body weights of male rats ranged from 150.1 to 185.8 g (group means, 170.6–171.4 g) and that of female rats 120.5–141.4 g (group means, 131.8–134.3 g).

No mortalities occurred during the study period. There were no signs of treatment-related effects during regular observations and during the battery of functional tests on day 83. The changes in body-weight gain in males (overall body-weight gain was only 3–6% lower than that of controls

at doses of ≥ 100 mg/kg bw per day) were minimal and not of biological significance. However, reductions in body-weight gain were observed in females at 100 mg/kg bw per day (very slightly but statistically significantly lower than concurrent controls from day 63 to termination) and at 250 mg/kg bw per day (from day 14 onwards and including the recovery period). Terminal body weights in females were reduced by 14% and 16% at 100 and 250 mg/kg bw per day, respectively.

There were no clear biologically significant treatment-related differences in the haematology of treated and control animals; reductions in concentrations of haemoglobin (3%) in males at the highest dose achieved statistical significance. Clinical chemistry showed statistically significant reductions in alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in males (11.4%, 19.3% and 19.6% respectively) at 250 mg/kg bw per day compared with controls, but the corresponding changes in females at the same dose (9.3%, 27.8% and 12.8% respectively) were not statistically significant. These observations were considered to be of no biological significance. Increases in blood albumin, total protein and potassium concentrations were observed at doses of 100 mg/kg bw per day in males and at 250 mg/kg bw per day in females compared with controls (Table 6). There was incomplete recovery from the clinical chemistry changes at the end of the recovery period. Urine analysis did not reveal any treatment-related changes.

Gross examination at necropsy did not reveal any treatment-related abnormalities. Organ weights showed a significant increase in the mean absolute and relative liver weights at doses of ≥ 100 mg/kg bw per day in males (17–33%) and females (9–29%) compared with controls (Table 6). A slight increase in absolute and relative kidney weights in males and in relative kidney weight in females only was observed at 250 mg/kg bw per day compared with controls. At the end of the recovery period, the absolute and relative liver weights were slightly increased in males at 250 mg/kg bw per day compared with controls. Histopathology at 13 weeks revealed slight centrilobular and midzonal hepatocellular hypertrophy with increased basophilia at doses of > 100 mg/kg bw per day in the 90-day group. Panlobular hepatocellular hypertrophy with increased basophilia and very slight individual cell hepatocellular necrosis were observed in males and females at the highest dose only. At the end of the 4-week recovery period, histopathology of the liver showed that 8 out of 10 male rats in the group at the highest dose exhibited very slight centrilobular hepatocellular hypertrophy with increased basophilia, indicating a reduction of the effects on the liver (Table 6).

The NOAEL was 10 mg/kg bw per day on the basis of reduction in body-weight gain, alterations in clinical chemistry, increase in absolute and relative liver weight and changes in the histopathology of the liver (Szabo, Campbell & Davis, 1992).

Table 6. Findings in a study in rats given diets containing quinoxyfen for 13 weeks followed by a recovery period

Finding	Dose (mg/kg bw per day)											
	Week 13								Week 17 (recovery)			
	Males				Females				Males		Females	
	0	10	100	250	0	10	100	250	0	250	0	250
Haemoglobin (g/dl)	15.1	14.7	14.8	14.6*	14.7	14.5	14.5	14.5	15.5	15.1*	15.2	15.1
Platelets ($\times 10^3$)	553	544	566	558	607	573	578	546*	564	588*	587	614*
Albumin (g/dl)	4.6	4.6	4.9*	5.0*	4.7	4.7	4.8	4.9*	4.9	5.0	4.8	4.6
Total protein (g/dl)	6.3	6.2	6.6*	6.7*	6.1	6.1	6.2	6.4*	6.5	6.8*	6.3	6.1*

ALP (U/l)	125	128	129	112*	97	90	103	88	120	112	93	87
ALT (U/l)	57	55	50	46*	54	50	51	39	56	41**	48	43
AST (U/l)	92	83	86	74*	94	90	89	82	98	75**	89	77*
Potassium (meq/l)	4.25	4.29	4.48*	4.60*	4.21	4.20	4.27	4.62*	4.10	4.17	4.44	4.39
Body weight (g)	284	285	280	276	173	169	166*	162*	300	296	183	173*
Liver weight (g)	7.46	7.80	8.57*	9.67*	4.48	4.42	4.68*	5.40*	8.0	8.7*	4.7	4.6
Liver (% body weight)	2.63	2.73	3.07**	3.50**	2.59	2.62	2.82*	3.35*	2.7	2.9*	2.6	2.7
Hypertrophy (centrilobular/ midzonal	0	0	10 +	3 +	0	0	3 +	0	0	8 ±	0	0
Hypertrophy (panlobular)	0	0	0	7 +	0	0	0	10 +	0	0	0	0
Necrosis (hepatocellular)	0	0	0	2 ±	0	0	0	9 ±	0	0	0	0

From Szabo, Campbell & Davis (1992)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; +, slight; ±, very slight.

* $p < 0.05$ by Dunnett's test, two-sided.

** $p < 0.05$ by Wilcoxon's test, two-sided.

Dogs

In a GLP-compliant study, conducted as a supplement to the 13-week dietary study, groups of two male and two female beagle dogs were fed diets containing quinoxyfen (purity, 98.7%) at nominal doses of 0 (control) or 250 mg/kg bw per day for 28 days. The parameters assessed were in-life observations, body weight and body-weight gain, feed consumption, haematology, clinical chemistry, urine analysis, organ weights, gross and histopathological evaluations. Blood samples for haematology and clinical chemistry were taken from fasted animals on days 13 and 24. Compound intake was determined from weekly assessments of feed consumption and body weight. Owing to unpalatability of the diet, the concentration in the calculated dietary intake was increased, resulting in increased unpalatability and lower food intake. The calculated weekly mean achieved dose was therefore affected and was 167–206 mg/kg bw per day for both sexes. However, the calculated mean for individual dogs showed a greater range and was 119–242 mg/kg bw per day for the 4-week period. Homogeneity and stability of quinoxyfen was shown to be acceptable in a previous 13-week dietary study in the dog (Wood & Szabo, 1992), hence no tests were conducted for this study.

There were no deaths or signs of toxicity during the study period. Ocular abnormalities were not observed in ophthalmological examinations. Slight (males) to marked (females) reduction in feed consumption was observed at 250 mg/kg bw per day. Consequently body-weight loss occurred in males (4.2% and 8.2%) and females (16.3% and 21.9%), while the controls gained in body weight.

Haematology, clinical chemistry and urine analysis and organ weight parameters did not show any clear treatment-related differences from values for controls. Gross examination of organs and tissues did not reveal any treatment-related changes. Histopathology of the liver showed slight vacuolation of the centrilobular and midzonal hepatocytes in treated animals. The vacuoles were of variable size and had a foamy appearance and were reported by the investigating laboratory to be suggestive of dilated endoplasmic reticulum rather than lipid accumulation. However, differential staining for lipids was not used or reported. This observation in the liver was noted to have been reported in an earlier investigation in dogs at doses of 500 and 1000 mg/kg bw per day (Szabo & Rachunek, 1992). There was no evidence of further treatment-related histopathological changes.

A NOAEL could not be determined primarily due to the reduction in food consumption, body-weight loss and histopathological changes in the liver at the nominal dose of 250 mg/kg bw per day (actual test dose, 119–250 mg/kg bw per day) (Szabo & Davis, 1993).

In a GLP-compliant study to determine palatability and potential toxicity, one male and one female beagle dog were given diets containing quinoxyfen (purity, 98.8%) at nominal doses of 0 (control), 100, 500 or 1000 mg/kg bw per day for 30 days. The parameters assessed were in-life observations, body weight and body-weight gain, feed consumption, haematology, clinical chemistry, urine analysis, organ weights, gross examination of organs and tissues and histopathological evaluations of the kidney, liver, testes and gross lesions. Body weights and feed consumption were determined weekly. At the start of treatment, the bodyweights ranged from 8.20–8.93 kg in males and 7.54–8.81 kg in females. The study was performed in accordance with OECD guideline No. 407.

The study design was based on a previous oral study (via capsules) in dogs (one male and one female per dose) with doses of 0–1000 mg/kg bw per day for 2 weeks (Weaver, 1988). In that study, body weight, feed consumption, electrocardiograms, enzyme induction, clinical chemistry, haematology and pathology of organs and tissues did not show any treatment-related changes.

In the present study, the achieved daily intakes of quinoxyfen were 86, 340 and 445 mg/kg bw per day in males and 130, 305 and 481 mg/kg bw per day in females at nominal doses of 100, 500 and 1000 mg/kg bw per day.

There were no mortalities or signs of toxicity during the study period. Feed consumption was reduced in all treated males and at doses of ≥ 500 mg/kg bw per day in females. The pattern of feed consumption was considered by the reporting laboratory to indicate a high degree of unpalatability. Body-weight loss was observed in males at doses of 500 (11%) and 1000 (35%) mg/kg bw per day and in females at doses of 500 (20%) and 1000 (30%) mg/kg bw per day compared with values for controls. Body-weight gain in males at 100 mg/kg bw per day was reduced (30%) compared with values for controls. There was a very slight statistically non-significant reduction in erythrocyte count, erythrocyte volume fraction and haemoglobin content in females at the highest dose compared with pre-test and control values. Clinical chemistry and urine analysis did not show any significant treatment-related changes. Organ weights were not reported. The testes in the male at the highest dose and the thymus in the male and female at the highest dose appeared to be small/atrophic on gross examination. However, microscopic examination of the testes did not reveal any abnormality. The main histopathological findings were slight multifocal hepatocellular necrosis in females at ≥ 500 mg/kg bw per day and slight (500 mg/kg bw per day) or moderate (1000 mg/kg bw per day) centrilobular and midzonal hepatocellular vacuolation in both sexes. Moderate multifocal vacuolation of the proximal convoluted tubule epithelium was observed in both sexes at 1000 mg/kg bw per day. Severe diffuse lymphoid depletion of the thymus was observed in both sexes at 1000 mg/kg bw per day.

The NOAEL was 86–130 mg/kg bw per day (100 mg/kg bw per day nominal dose) on the basis of body-weight loss and histopathological changes in the liver [including slight multifocal hepatocellular necrosis] at 305–340 mg/kg bw per day (nominal dose, 500 mg/kg bw per day). Impaired body-weight gain and food consumption in the male dog at 100 mg/kg bw per day was considered to result from unpalatability and was therefore of limited toxicological relevance in setting the NOAEL (Szabo & Rachunek, 1992).

Groups of four male and four female beagle dogs were fed diets containing quinoxyfen (purity, 98.7%) at nominal doses of 0 (control) 10, 50 or 100 mg/kg bw per day for 90 days. The parameters assessed were in-life observations, body weight and body-weight gain, feed consumption, haematology, clinical chemistry, urine analysis, organ weights, gross and histopathological evaluations. The achieved mean daily compound intakes were 10, 50 and 100 mg/kg bw per day in males and 10, 50 and 101 mg/kg bw per day in females at nominal doses of 10, 50 and 100 mg/kg bw per day. At the start of treatment, body weights ranged from 8.19 to 10.82 kg in male dogs and 7.48–9.30 kg in female dogs.

No mortalities occurred during the study period. There were no adverse effects on animal behaviour. Ophthalmological examinations at the beginning and on day 87 of the study did not reveal any treatment-related ocular defects. A slight transient reduction in food consumption was observed

in the highest dose males but the animals adapted to the initial unpalatability. However, it was noted that females at the highest dose consumed more feed than did controls. Body-weight gain was not affected. Clinical chemistry, urine analysis and haematology parameters did not show any biologically significant treatment-related changes.

The terminal body weights and organ weights did not show any significant treatment-related intergroup differences; increased relative liver weights in the groups at 50 mg/kg bw per day were not reproduced at 100 mg/kg bw per day. Gross examination at necropsy did not reveal any significant treatment-related changes. Histopathological examination revealed only a single incidence of slight and midzonal centrilobular hepatocellular hypertrophy, with no associated clinical chemistry or additional histopathology findings, which indicated that the dose of 100 mg/kg bw per day may represent a threshold dose for histopathological effects on the liver (Table 7).

The NOAEL was 100 mg/kg bw per day. The single occurrence of hepatocellular hypertrophy, with no associated clinical chemistry or other histopathological findings, was not considered to be adverse (Wood & Szabo, 1992).

Table 7. Hepatic findings in dogs fed diets containing quinoxyfen for 13 weeks

Finding	Dose (mg/kg bw per day)							
	Male				Female			
	0	10	50	100	0	10	50	100
Body weight (g)	11495	10813	10903	11773	9943	9585	9373	9575
Liver weight (g)	314	315	338	339	263	255	296	284
Liver (% bw)	2.73	2.91	3.13*	2.90	2.66	2.67	3.16*	2.98
(range)	(2.6–2.9)	(2.8–3.2)	(2.8–3.6)	(2.5–3.4)	(2.4–3.1)	(2.3–3.0)	(2.8–3.4)	(2.7–3.5)
Centrilobular & midzonal hepatocyte hypertrophy	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4

From Wood & Szabo (1992)

* $p < 0.05$

In a GLP-compliant study, groups of four male and four female beagle dogs were fed diets containing quinoxyfen (purity, 96.2%) at a dose of 0 (control) 5, 20 or 200 mg/kg bw per day for 52 weeks. The parameters assessed were in-life observations, ophthalmological examinations, body weight and body-weight gain, feed consumption, haematology, clinical chemistry, urine analysis, selected organ weights, gross examinations of organs and tissues and histopathological evaluations.

The mean achieved compound intakes were 6, 20 and 194 mg/kg bw per day in males and 5, 21 and 203 mg/kg bw per day in females at nominal doses of 5, 20 and 200 mg/kg bw per day respectively. At the start of treatment, the dogs were aged 7 months and body weights ranged from 10.4 to 12.1 kg (group means, 11.0–11.3 kg) in male dogs and from 7.8 to 8.8 kg (group means, 8.2-8.3 kg) in female dogs.

One male dog at 200 mg/kg bw per day died on day 62 with symptoms suggestive of aspiration pneumonia, but a severe systemic disease consistent with canine parvovirus infection was also present. Decreased activity, pale mucous membranes, dehydration, weakness and in addition, blood, vomitus, runny and watery stools in the cage were observed before death. A second dog at 200 mg/kg bw per day was humanely killed on day 119 after evidence of treatment-related anaemia and weight loss (approximately 2 kg). Eye examinations performed before the start of treatment and 1 week before termination did not reveal any treatment-related findings. Feed consumption was variable but generally reduced in male and female dogs at 200 mg/kg bw per day compared with dogs in the

control group. This was observed from the first week of treatment and was considered to be suggestive of very slight unpalatability at the highest dose. It was noted that this did not affect the achievement of target doses. Body-weight gain was affected and the body weights of males and females at the highest dose were lower than those of controls throughout the study. The mean terminal body weights were reduced in males (14.5%) and females (9.25%) at 200 mg/kg bw per day compared with those of dogs in the control group.

Haematological tests at 3, 4, 6, 7, 8 and 12 months showed marked reductions in haemoglobin concentration, erythrocyte volume fraction, erythrocyte and leukocyte counts in one male dog at 200 mg/kg bw per day at 3 and 4 months compared with pre-study and inter/intra-group values. Peripheral blood smears from this dog, which was killed and necropsied on day 119, showed marked polychromasia and moderate hypochromasia after 3 months and additionally slight anisocytosis on day 119. A female dog at 200 mg/kg bw per day also showed reductions in haemoglobin concentration, erythrocyte volume fraction and erythrocyte counts from 3 months onwards. Microscopic examination of peripheral blood smears revealed slight hypochromasia. However, there was apparent compensation of anaemia in the female dog and the unscheduled haematological assays were discontinued after 8 months. Extramedullary haematopoiesis was seen in most dogs at 200 mg/kg bw per day.

Clinical chemistry showed an increase (greater than twofold) in serum ALP activity in male and female dogs at 200 mg/kg bw per day compared with controls. The magnitude of the increase in ALP exhibited a clear relationship with duration of dosing. Increased serum ALP activity might be indicative of cholestasis (impaired hepatobiliary function), enzyme induction or hepatocellular damage. Transient or non dose-related changes in bilirubin and cholesterol were considered to be not treatment-related. Urine analysis at 6 months and at necropsy did not reveal any significant treatment-related changes.

The absolute (27.5% in males and 9.4% in females) and relative liver weights of male and female dogs at 200 mg/kg bw per day were increased compared with controls and were considered to be treatment-related (Table 8). The remaining changes in relative organ weights of the brain, kidney and pituitary in dogs at 200 mg/kg bw per day were considered to be related to the lower body weight of this group and, in the absence of any supporting histopathological or functional evidence in these organs, not treatment-related.

Histopathology of the liver showed an increase in the incidence of diffuse hepatocyte hypertrophy (three out of four males and three out of four females) and increased bile in bile canaliculi (one out of four males and one out of four females) in both sexes at 200 mg/kg bw per day. No treatment-related changes were observed in the kidneys and testes.

The NOAEL was 20 mg/kg bw per day on the basis of reduced food consumption and body-weight gain, haematological changes consistent with haemolytic anaemia and a compensatory response, increased serum activity of ALP, increased liver weight and histopathological changes in the liver at 200 mg/kg bw per day (Cosse et al., 1995).

Table 8. Hepatic findings in dogs[a] fed diets containing quinoxyfen for 52 weeks

Finding	Dose (mg/kg bw per day)							
	Male				Female			
	0	5	20	200[b]	0	5	20	200
Body weight (g)	12923	12735	13274	11171	9924	9187	9688	8249
Liver weight (g)	296	372	369	407	269	271	279	297
Liver (% bw)	2.3	2.9	2.8	3.6	2.7	2.9	2.9	3.7

Liver, extramedullary haematopoiesis	1	0	0	2	1	1	0	3
Increased hepatocyte size (slight)	0	0	0	3 (n = 4)	0	0	0	3
Splenic extramedullary haematopoiesis	0	0	0	1 (n = 4) (moderate)	0	1 (very slight)	0	2 (slight)
Erythrocyte volume fraction								
At 3 months	0.513	0.529	53.4	0.467 (n = 3)	0.527	0.551	0.547	0.499
At 12 months	0.478	0.467	0.511	0.489	0.526	0.481	0.486	0.474
ALP (mU/ml)								
At 3 months	55	53	51	90	52	56	72	90
At 12 months	41	43	44	136*	46	59	50	99*

From Cosse et al. (1995)

ALP, alkaline phosphatase.

[a] n = 4.

[b] n = 2.

* $p < 0.05$

(b) Dermal administration

In a GLP compliant study, groups of 10 male and 10 female Fischer 344 rats received quinoxyfen (purity, 97.4%; lot DECO-97-152-1) as 20 daily dermal doses at 0, 10, 100 or 1000 mg/kg bw per day in 0.5% methylcellulose, applied to clipped skin, under occluded dressing for 6 h and on 5 days per week for 4 weeks. Routine investigations of body weight, food consumption, haematology, clinical chemistry, urine analysis and ophthalmoscopy were performed. Gross pathological examinations were performed on a wide range of tissues from all animals; histopathological examinations were performed on all tissues from rats in the control group and at the highest dose, plus liver, kidney and lung from rats at the lowest and intermediate doses. There were no adverse findings associated with administration of quinoxyfen; several sporadic findings did not exhibit any dose–response relationship or were within rages for historical controls (e.g. testes weights) and were not considered to be adverse. The NOAEL was 1000 mg/kg bw per day, the highest dose tested, equal to 714 mg/kg bw per day averaged over the study duration (Baker & Yano, 2000).

(c) Inhalational administration

No data were available.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a GLP-compliant study of carcinogenicity in mice, groups of 50 male and 50 female Crl:CD-1(ICR)BR mice received diets containing quinoxyfen (purity, 97.4%) at variable concentrations to give nominal doses of 0 (control), 20, 80 or 250 mg/kg bw per day for 80 weeks. In-life observations, feed consumption, body-weight gain and mortalities were recorded throughout the study. Haematological tests were performed in weeks 52 and 80 with samples taken from 10 mice/sex from the control group and the group at 250 mg/kg bw per day. All surviving animals were killed at the end of the scheduled treatment period and tissues and organs were examined macroscopically and microscopically.

The homogeneity and stability of quinoxyfen in the diet were shown to be acceptable. The dietary concentrations of quinoxyfen taken at 3-monthly intervals were within 9% of the nominal doses. The mean achieved intakes of quinoxyfen were 20, 80 and 250 mg/kg bw per day in males and 20, 81 and 251 mg/kg bw per day in females. At the start of the study, the mean body weights were 24.3–24.9 g in males and 21.8–22.3 g in females.

Mortalities during the 80 weeks of treatment were 40, 34, 22 and 26% in males and 24, 20, 22 and 18% in females at doses of 0, 20, 80 and 250 mg/kg bw per day, respectively. There were no treatment-related intergroup differences in the incidence of mortalities. In-life observations including the incidence of palpable masses did not reveal any treatment-related effects. There were no significant treatment-related differences in feed consumption between treated and control animals. However, variable reductions in body-weight gain were observed in males (6–11%) and females (9–20%) at 250 mg/kg bw per day. Haematology did not reveal any significant treatment-related intergroup differences. There was a statistically significant increase in the relative liver (16%) and kidney (12%) weights in females receiving 250 mg/kg bw per day compared with controls. The patterns of non-neoplastic lesions were typical of aged mice. There were no statistically significant increases in macroscopic or microscopic findings in mice exposed to quinoxyfen. A relatively high incidence of uterine benign endometrial polyps was noted in females at the lowest dose (4 out of 50 versus 0 out of 50 in the controls) but there was no evidence of a dose–response relationship and this finding was not considered to be treatment related. The incidence of liver tumours was similar in control and treated groups. Quinoxyfen was not tumourigenic in mice under the test conditions of this study.

The overall NOAEL was 80 mg/kg bw per day on the basis of reduction in body-weight gain in males and increase in the relative liver and kidney weights in females at 250 mg/kg bw per day. The NOAEL for carcinogenicity was 250 mg/kg bw per day, the highest dose tested (Bellringer, 1995).

Rats

In a GLP-compliant study, groups of 50 male and 50 female Fischer 344 rats were fed diets containing quinoxyfen (purity, 96.2–97.4%) at variable concentrations to give nominal doses of 0 (control), 5, 20 or 80 mg/kg bw per day for up to 24 months. Additionally, satellite groups of 15 male and 15 female rats were treated similarly and killed after 12 months. The rats in the satellite group were divided into two subgroups of five (exclusively for neuropathology) or 10 rats of each sex for the assessment of neurotoxicity, clinical laboratory tests (haematology, clinical chemistry and urine analysis) and parameters at interim kill. The findings relating to neurotoxicity are presented here separately; Shankar et al., 1995. In-life observations, feed consumption, body-weight gain and mortalities were recorded throughout the study. Haematology, clinical chemistry and urine analysis were measured at 6 and 12 months using samples taken from 10 males and 10 females per dose from the satellite groups and at 18 and 24 months from 10 and 20 rats respectively of the main-study group. All surviving animals were killed at the end of the scheduled treatment period and an extensive set of tissues and organs was examined macroscopically and microscopically.

Homogeneity and stability of quinoxyfen in the diet was shown to be acceptable. Mortalities were 30, 34, 36 and 36% in males and 20, 20, 16 and 24% in females at doses of 0, 5, 20 and 80 mg/kg bw per day respectively. There were no treatment-related intergroup differences in the incidence of mortalities. The causes of death or moribundity were noted by the investigating laboratory to be typical for the Fischer rat. In-life observations including the incidence of palpable masses did not reveal any clear treatment-related effects. A slight increase in the incidence of perineal soiling in females at 80 mg/kg bw per day was noted (7 out of 65, 10 out of 65, 4 out of 65 and 16 out of 65 at doses of 0, 5, 20 and 80 mg/kg bw per day respectively). Feed consumption was consistently lower in male rats at 80 mg/kg bw per day from day 418 to the end of the study. In female rats, feed consumption was generally lower than that of controls during the first 70 days and intermittently during the remainder

of the treatment period. The mean body-weight gain was reduced by 9.1% (4.1–13.9%) in males and 9.7% (3.8–12.5%) in females at 80 mg/kg bw per day compared with controls. The investigating laboratory suggested that reduced body-weight gain in the second year was probably due to the increased incidence of chronic progressive glomerulonephropathy, a spontaneous disease common among aging laboratory rats (Table 9).

Haematology and urine analysis parameters did not show any significant treatment-related differences. Differences in the clinical chemistry of treated males compared with concurrent controls included an increase in blood urea nitrogen at 80 mg/kg bw per day at 18 and 24 months and a reduction in ALT at 80 mg/kg bw per day after 24 months. In females, there was an increase (18%) in cholesterol at 80 mg/kg bw per day. An apparent increase in serum triglyceride concentrations in males at the intermediate and highest dose at 24 months was traced back to a small number of animals with outlying values; triglyceride levels at 18 months were similar in all groups. The other differences identified were either within the range for historical controls, not consistently observed or did not show a dose–response relationship and were considered to be of limited toxicological relevance. The clinical chemistry alterations seen in the 13-week study in rats were not reproduced in this study.

In the rats killed after 12 months there was an increase in the absolute and relative liver weight in males and females at 80 mg/kg bw per day (Table 9). A slight statistically significant increase in the absolute (8%) and relative (11%) kidney weight was observed in males at 80 mg/kg bw per day compared with controls, but in females the increase was not statistically significant. Gross examination of organs and tissues did not reveal any treatment-related changes. Histopathological examination of tissues and organs revealed an increase in the incidence of slight hepatocellular hypertrophy (8 out of 10) in male rats at 80 mg/kg bw per day compared with controls (1 out of 10).

After 24 months, there was an increase in the absolute and relative liver weight at doses of ≥ 20 mg/kg bw per day, but with no dose–response relationship; an increase in the absolute and relative weight of the testes at 80 mg/kg bw per day and an increase in the relative kidney weight in males compared with controls. In females, organ-weight differences at 24 months were increases in the relative liver and kidney weights at 80 mg/kg bw per day compared with controls. Histopathology revealed a higher incidence of moderate chronic progressive glomerulonephropathy in males at 80 mg/kg bw per day. There was no evidence of treatment-related histopathological changes in the liver at 24 months.

Table 9. Findings in a study in rats fed diets containing quinoxyfen for up to 24 months

Finding	Dose (mg/kg bw per day)							
	Male				Female			
	0	5	20	80	0	5	20	80
Body weight (g)								
At 18 months	422	416	426	406*	256	267	258	246
At 24 months	354	345	351	335*	264	271	261	245*
Liver weight (g) at 24 months	11.8	12.0	13.1*	12.6	7.5	7.8	7.4	7.5
Liver (% bw)	3.4	3.5	3.8*	3.8*	2.9	2.9	2.9	3.1
Kidney weight (g) at 24 months	3.0	3.0	3.2	3.0	2.0	2.0	2.0	2.0
Kidney (% bw)	0.87	0.87	0.92	0.90*	0.78	0.75	0.78	0.83*

Testes weight (g) at 24 months	5.4	5.8	6.9	8.0*	—	—	—	—
Triglyceride (mg/dl) at 18 months	116	115	118	143	118	133	109	99
Cholesterol (mg/dl) at 18 months	156	155	156	170	163	162	169	192*
ALT (mU/ml) at 24 months	41 ± 9	43 ± 30	49 ± 39	34* ± 11	35 ± 7	34 ± 7	41 ± 24	32 ± 6
BUN (mg/dl) at 18 months	16 ± 1	16 ± 1	16 ± 2	18* ± 2	16 ± 1	16 ± 2	17 ± 2	17 ± 3
ALP (mU/ml) at 18 months	51 ± 7	56 ± 14	316 ± 833	60 ± 31	39 ± 6	40 ± 4	41 ± 5	39 ± 5
Glomerulonephropathy (moderate or greater) at 24 months	19	16	21	37*	1	0	2	2
Hepatocyte increased size at 12 months	1/10	1/10	0/10	8/10	0/10	0/10	0/10	0/10
Leydig cell tumours at 12 months	7/10	1/1	0/1	9/10	—	—	—	—
Leydig cell tumours at 24 months	50	46	47	49	—	—	—	—

From Redmond et al. (1995)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

* $p < 0.05$.

Quinoxyfen was not tumourigenic in the rat. All males in the control group had Leydig cell tumours at 24 months, and the incidence in the control group at 12 months was 7 out of 10, making it difficult to determine whether there was any effect of administration of quinoxyfen.

The NOAEL was 20 mg/kg bw per day on the basis of reduced body-weight gain, slight clinical chemistry changes consistent with the liver and kidney findings, increase in kidney and liver weights and histopathological changes in the liver (at 12 months only) and the kidney at 80 mg/kg bw per day. Because of the transient nature of the histopathological changes in the liver at 80 mg/kg bw per day (increase in the incidence of slight hepatocellular hypertrophy in male rats at 12 but not 24 months), and absence of further evidence of liver toxicity, the increase in liver weight at 20 mg/kg bw per day was considered to be of limited toxicological significance (Redmond et al., 1995).

2.4 Genotoxicity

Quinoxyfen was not mutagenic or clastogenic in three tests for genotoxicity in vitro in the presence or absence of metabolic activation, nor in a test for micronucleus formation in mice in vivo (Table 10). Positive controls adequately demonstrated the sensitivity of the assays. All studies were compliant with GLP.

The Meeting concluded that quinoxyfen was unlikely to be genotoxic.

Table 10. Results of studies of genotoxicity with quinoxyfen

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium strains* TA98, TA100, TA1535, TA1537	10–5000 µg/plate (precipitate at >500µg/plate)	97.4	Negative +S9 Negative −S9	Xu (1993)
Chromosome aberrations	Rat lymphocytes	3.1–100 µg/ml (25–100 µg/ml scored)	97.4	Negative +S9 Negative −S9	Linscombe & Lick (1994)
Gene mutation	Chinese hamster ovary cells (CHO-K1, BH4) at *Hprt* locus	2.5-80 µg/ml	97.4	Negative +S9 Negative −S9	Pant (1994)
In vivo					
Micronucleus formation	Bone marrow from CD-1 mice (five of each sex per group per time-point)	1250, 2500 or 5000 mg/kg bw by gavage (sacrificed at 24, 48 or 72 h)	97.4	Negative	Gollapudi & Lick (1994)

S9, 9000 × *g* supernatant from livers of male Sprague-Dawley rats.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a GLP-compliant study, groups of 30 male and 30 female Sprague-Dawley rats were given diets containing quinoxyfen at variable concentrations to provide nominal doses of 0 (control), 5, 20 or 100 mg/kg bw per day over two generations. P_1 rats (aged 6 weeks) received quinoxyfen for 10 weeks before mating (1 : 1), throughout mating, gestation and lactation. The P_1 dams were allowed to rear the F_{1a} pups for 3 weeks before weaning. Thirty weanlings of each sex per group were randomly selected from the F_{1a} to constitute the P_2 generation. Two weeks after weaning of the F_{1a}, the P_1 rats were remated to produce the F_{1b} offspring. The P_2 parents were mated to produce the F_2 litter. At the time of weaning of the F_{1a}, F_{1b} and F_2 litters, 10 pups of each sex per group were randomly selected and necropsied. Achieved intakes varied throughout the different phases of the study, but to a lesser extent than is normal with a study of reproduction; mean intakes over the entire study were 0, 6, 22 and 110 mg/kg bw per day for males and 0, 8, 28 and 141 mg/kg bw per day for females.

There were no treatment-related mortalities or signs of toxicity in P_1 and P_2 adults or F_{1a}, F_{1b} and F_2 pups. Slightly reduced feed consumption was noted in the group at 100 mg/kg bw per day at the end of lactation in P_1/F_{1a}, P_1/F_{1b} and P_2/F_2 adult females and litters. This finding was considered to be probably a result of lower feed consumption in the pups at 100 mg/kg bw per day rather than in dams. However, there were no treatment-related intergroup differences in body-weight gain in dams. There were no treatment-related differences in reproductive indices and pup survival in the P_1/F_{1a}, P_1/F_{1b} and P_2/F_2 adults and litters (Table 11). There were no treatment-related differences in litter size or birth weights, but slight reductions in the mean pup body weight were observed in the F_{1a}, F_{1b} and F_2 litters at 100 mg/kg bw per day at the end of lactation, which recovered post-weaning (Table 11). Gross examinations at necropsy did not reveal any treatment-related changes in adults or pups. Histopathology of tissues and organs revealed very slight centrilobular hepatocellular hypertrophy in male P_1 rats (14 out of 30 compared with 1 out of 28 controls) and very slight (25 out of 30) or

slight (3 out of 30) hepatocellular hypertrophy in male P_2 rats compared with 0 out of 30 concurrent controls. No clinical chemistry assays were performed in this study. Slight, bilateral inflammation of the epididymis was increased at 100 mg/kg bw per day in parental males of both generations, relative to concurrent controls, but was within the range for historical controls; epididymides were not investigated histopathologically at 20 or 5 mg/kg bw per day.

Table 11. Findings in a two-generation study of reproductive toxicity in rats given diets containing quinoxyfen

Finding	Nominal dose (mg/kg bw per day)			
	0	5	20	100
F_{1d}/F_{1b}				
Litter size day 1	15 / 14	14 / 16	14 / 16	15 / 17
Litter size day 21	14 / 14	13 / 15	14 / 14	14 / 15
Pup weight day 0 (g)	6.7 / 6.9	7.1 / 6.9	6.7 / 6.9	6.6 / 6.7
Pup weight day 21 (g)	43 / 41	47* / 40	42 / 41	39* / 37
F_{1a}–P_2 bw, day 83, M/F (g)	553 / 311	561 / 341*	581 / 338*	547 / 315
F_2				
Litter size day 1	14	15	14	13
Litter size day 21	13	15	13	12
Pup weight day 0 (g)	6.7	6.9	6.7	6.6
Pup weight day 21 (g)	42	43	41	40

From Liberacki et al. (1995)

* $p < 0.05$

The NOAEL for general toxicity and pup development was 20 mg/kg bw per day on the basis of slight but consistent reduction in body-weight gain in pups during lactation and hepatocyte hypertrophy in the liver of parental males, without investigations on serum enzyme activity. The NOAEL for reproductive performance was 100 mg/kg bw per day as there were no adverse effects on reproductive performance at the highest test dose (Liberacki et al., 1995).

(b) Developmental toxicity

Rats

In a GLP-compliant study, groups of 30 mated specific pathogen free (SPF) female Crl:CD(SD)BR rats (body weight, 202–239 g) were given quinoxyfen (purity, 97.4%) at a dose of 0 (control), 100, 300 or 1000 mg/kg bw per day suspended in 1% methyl cellulose by gavage from day 6 to 15 of presumed gestation. Dams were killed on day 20 and the pups were delivered by caesarean section. The liver, kidneys, gravid uterine weights, and the number of corpora lutea, implantations, resorptions and dead and live fetuses were recorded. Fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There was a single mortality at 1000 mg/kg bw per day that was the result of an intubation error. The number of pregnant dams was 24, 27, 28 and 28 at doses of 0 (control), 100, 300 and 1000 mg/kg bw per day. No signs of toxicity or changes in animal behaviour were observed during the dosing period. Food consumption and body-weight gain were not affected by the treatment. At termination, no gross abnormalities were observed in dams. There were no significant treatment-related effects on the number of litters with external, visceral or skeletal effects. A raised incidence of pups with extra ribs was seen at the highest dose (13% vs 8–9% in other groups) but was within the range for historical controls (7–22%) and was not clearly treatment related.

The NOAEL for maternal toxicity, fetotoxicity and teratogenicity was 1000 mg/kg bw per day, the highest dose tested (Brooker, 1994).

Rabbits

In a preliminary, GLP-compliant study to determine appropriate doses for a study of gavage, groups of seven mated New Zealand White rabbits were given quinoxyfen (purity, 96.2%) at a dose of 0 (control), 100, 300, 600 or 1000 mg/kg bw per day suspended in 0.5% methyl cellulose by gavage from day 7 to day 19 of presumed gestation. Dams were killed on day 20 and the pups were delivered by caesarean section. The liver and kidney weights, and the number of corpora lutea, implantations, resorptions, and the number of dead or live fetuses were recorded. Fetuses were not weighed or sexed, but were examined for gross external abnormalities.

At the start of the study, the female rabbits were aged 5–6 months and body weights ranged from 2.4 to 4.0 kg. Analysis of all dose suspensions showed mean concentrations of quinoxyfen of 88–127%. Analysis of samples taken from three separate layers of the least and most concentrated suspensions showed adequate homogeneity.

All females at 600 and 1000 mg/kg bw per day were killed humanely on day 15 of gestation and data on reproductive parameters were not recorded for these two groups. The number of pregnant dams was 5 out of 7, 4 out of 7 and 6 out of 7 at doses of 0 (control), 100 and 300 mg/kg bw per day. At 300 mg/kg bw per day, one rabbit died and two were killed humanely. Signs of toxicity in the two rabbits that were killed and in rabbits at the higher doses were body-weight loss, marked inanition and reduced faecal output. The remaining rabbit at 300 mg/kg bw per day that died spontaneously did not exhibit any prior signs of toxicity. Reduced feed consumption was observed in animals at doses of ≥ 300 mg/kg bw per day compared with controls during the dosing period resulting in reduced body-weight gains. There were no treatment related changes in pregnancy rates, numbers of corpora lutea, implantations, resorptions or litter size in rabbits at 100 or 300 mg/kg bw per day. There was a significant increase in the relative liver weight in rabbits at 300 mg/kg bw per day.

The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of evidence of systemic toxicity at ≥ 300 mg/kg bw per day. There was no evidence of fetotoxicity or teratogenicity at 300 mg/kg bw per day (Zablotny et al., 1993).

In the main, GLP-compliant study, groups of 18 mated New Zealand White rabbits were given quinoxyfen (purity, 96.2%) at a dose of 0 (control), 20, 80 or 200 mg/kg bw per day suspended in 0.5% methyl cellulose by gavage from day 7 to day 19 of presumed gestation. Dams were killed on day 28 and the pups were delivered by caesarean section. The liver, kidneys, gravid uterine weights, and the number of corpora lutea, implantations, resorptions and dead and live fetuses were recorded. Fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities. At the start of the study, the body weights of the female rabbits ranged from 2.4 to 3.5 kg. The mean concentration of quinoxyfen in the dosing suspensions ranged from 91% to 105% of the target concentrations. The suspensions were homogenous.

There were no treatment-related mortalities during the study. One dam at the highest dose died as a result of an error during gavage. Signs of toxicity observed in dams at 200 mg/kg bw per day included decreased faecal output, soft faeces, perineal soiling, blood or urine containing blood in cage pan and abortions. The number of pregnant dams was 15, 14, 15 and 14 at doses of 0 (control), 20, 80 and 200 mg/kg bw per day. Abortions were observed in two dams at 20 mg/kg bw per day and in five dams at 200 mg/kg bw per day. The abortions at 20 mg/kg bw per day were considered to be probably not treatment-related because there were no abortions at the higher dose of 80 mg/kg bw per day and the incidence of abortion was noted to be close to the upper limit for historical controls. Dams which aborted in the 200 mg/kg bw per day did so on days 20–27 of gestation, having shown

marked inanition, decreased faecal output and body-weight loss; these abortions are considered to be treatment-related. Reduction in food consumption was observed in females of the 200 mg/kg bw per day from days 13–22. There were no significant treatment-related differences in the reproductive and fetal parameters including, pregnancy rate, the number of corpora lutea, implantations, viable fetuses per litter, percentage implantation loss, resorption rates, fetal sex ratios, fetal body weights or gravid uterus rates, or in the incidence of fetal abnormalities.

The NOAEL for maternal toxicity was 80 mg/kg bw per day on the basis of reduction in food consumption, reduction in maternal body-weight gain, inanition and increased incidence of abortions at 200 mg/kg bw per day. Quinoxyfen was not fetotoxic or teratogenic in rabbits under the conditions of this study. Abortions were noted to occur in the presence of overt maternal toxicity and were therefore not considered to indicate direct fetal toxicity (Zablotny et al., 1994).

2.6 Special studies

(a) Induction of liver enzymes

In an assay for induction of liver enzymes in mice, groups of five male and five female CD-1 mice received diets containing quinoxyfen at fixed dietary concentrations of 0, 100, 400, 1500, 6000 or 12000 ppm for 2 weeks. Observations included signs of toxicity, body weights, haematology, clinical chemistry, organ weights and gross and microscopic pathology. Hepatic mixed function oxidase activity was evaluated using the enzymes *p*-nitroanisole *O*-demethylase, 7-ethoxyresorufin *O*-deethylase, benzphetamine *N*-demethylase and hepatic cytochrome P-450. This study was not certified to comply with GLP.

One mouse at 1500 ppm died on day 4. Body-weight gain was reduced transiently at the start of exposure at 12000 ppm; food consumption was not measured. There was no other significant effect on growth. The activity of serum ALP and ALT, primarily indicators of effects on the liver, was significantly increased in males at the highest dose (Table 12). Absolute and relative liver weights were significantly increased in males and females at 6000 and 12000 ppm (Table 12). These animals were reported as having varying degrees of centrilobular hepatocyte hypertrophy (actual data not presented).

Significant increases in activity of the hepatic mixed function oxidase enzymes (enzymes *p*-nitroanisole *O*-demethylase, 7-ethoxyresorufin *O*-deethylase, benzphetamine *N*-demethylase) and increased P-450 content occurred in one or both sexes at 1500, 6000 and 1200 ppm (Table 12). These data indicated that quinoxyfen is an inducer of mixed function oxidase cytochrome P-450 isoenzymes at dietary concentrations of 1500 ppm and greater after 2 weeks of exposure.

The NOAEL for liver enzyme induction in the mouse was 400 ppm, equivalent to 55 mg/kg bw per day (Weaver, 1989).

Table 12. Hepatic findings in mice fed diets containing quinoxyfen for 2 weeks

Finding	Dietary concentration (ppm)					
	0	100	400	1500	6000	12000
Males						
Relative liver weight (% bw)	3.8	3.9	4.0	4.1	4.9*	5.3*
p-Nitroanisole *O*-demethylase activity (nmol/mg/h)	25	24	23	31*	41*	42*
7-Ethoxyresorufin *O*-deethylase activity (nmol/mg/h)	7.7	8.0	6.7	9.6	10.2	12.9*
Benzphetamine *N*-demethylase activity (nmol/mg/h)	57	49	46	70	101*	114*
ALT activity (IU/l)	17	20	22	22	29	49*
Alkaline phosphatase activity (IU/l)	115	134	152	205	138	235*

Microsomal P450 (nmol/mg)	0.9	1.0	0.9	1.2*	1.2*	1.4*
Females						
Relative liver weightt (% bw)	3.9	4.0	3.9	4.3	5.7*	7.0*
p-Nitroanisole *O*-demethylase activity (nmol/mg/h)	27	28	31	39*	48*	56*
7-Ethoxyresorufin *O*-deethylase activity (nmol/mg/h)	6.3	7.8	8.4	13.0*	18.1*	21.0*
Benzphetamine *N*-demethylase activity (nmol/mg/h)	84	80	82	116	146*	191*
ALT activity (IU/l)	40	24	25	27	41	44
Alkaline phosphatase activity (IU/l)	259	308	283	237	361	259
Microsomal P450 (nmol/mg)	0.7	0.7	0.8	1.0*	1.2*	1.4*

From Weaver (1989)

ALT, alanine aminotransferase.

* $p < 0.05$.

In a study on induction of liver enzymes in rats, groups of five male and five female Fischer 344 rats received diets containing quinoxyfen at a concentration of 0, 100, 500, 2500, 12500 or 25000 ppm for 2 weeks. Observations included signs, body weights, food consumption, haematology, clinical chemistry, organ weights and gross and microscopic pathology. Activity of hepatic mixed function oxidase was evaluated using only one marker, the enzyme *p*-nitroanisole *O*-demethylase. This study was not certified to comply with GLP.

Two males at the highest dose and all females at the highest dose died, hence no data were presented for this dietary concentration. From the initiation of treatment, dose-dependent body-weight loss occurred at the two higher dietary concentrations. This was associated with correspondingly marked reductions in food consumption. There were no toxicologically important effects on haematology and clinical chemistry parameters, a slight increase in serum bilirubin and alkaline phosphatase activity in females at 12500 ppm might have been indicative of early hepatic damage. Relative liver weights were increased in males and females at 2500 ppm and 12500 ppm (Table 13). There were no specific histopathological effects attributable to treatment with quinoxyfen.

Slight increases in *p*-nitroanisole *O*-demethylase activity were evident for females at 2500 ppm. Significant increases in *p*-nitroanisole *O*-demethylase were observed in males and females at 12500 ppm (Table 13). These data indicated that quinoxyfen is an inducer of P-450 enzymes at dietary concentrations of 2500 ppm and greater after 2 weeks of exposure.

The NOAEL for xenobiotic enzyme induction in rats exposed to quinoxyfen for 2 weeks was 500 ppm, equal to 45 mg/kg bw per day (Weaver, 1988).

Table 13. Hepatic findings in rats fed diets containing quinoxyfen for 2 weeks

Finding	Dietary concentration (%)				
	0	0.01	0.05	0.25	1.25
Males					
Relative liver weightt (% bw)	3.1	3.1	3.1	3.6*	4.0*
p-Nitroanisole *O*-demethylase activity (nmol/mg/h)	22	22	24	25	47*
Alkaline phosphatase activity (IU/l)	336	376	341	368	368
Bilirubin (mg/dl)	0.07	0.07	0.07	0.08	0.13

Females					
Relative liver weightt (% bw)	3.1	3.2	3.2	3.4*	5.5*
p-Nitroanisole *O*-demethylase activity	17	17	19	24*	48*
Alkaline phosphatase activity (IU/l)	283	304	298	285	355*
Bilirubin (mg/dl)	0.06	0.06	0.06	0.12	0.16*

From Weaver (1988)

* $p < 0.05$.

(b) Neurotoxicity

(i) Acute neurotoxicity

In a GLP-compliant study of acute neurotoxicity, groups of 10 male and 10 female Fischer F344 rats received a single dose of quinoxyfen (purity, 97.4%) at 0, 200, 632 or 2000 mg/kg bw by gavage in 0.5% methyl cellulose. The concentration, homogeneity and stability of quinoxyfen solutions were analysed. Food, water and housing conditions were controlled and monitored throughout the study. Clinical observations for acute effects were made before dosing and daily during days 1–4. Body weights were recorded before dosing and on days 1, 2, 8 and 15. Neurobehavioural assessments, including functional observational battery (FOB) hand-held and open-field observations, grip performance, landing foot splay and rectal temperature) and motor activity assay, were conducted before exposure and on days 1, 8 and 15. Ophthalmologic examinations were conducted before dosing and at termination. At necropsy on day 16, organs and tissues from all animals were examined grossly. Five males and five females from each group were selected for evaluation of neuropathology, and sections of central and peripheral nervous tissues from perfusion-fixed rats at 0 and 2000 mg/kg bw per day were assessed histopathologically.

Ophthalmology did not reveal any treatment-related change. Treatment with quinoxyfen did not adversely affect cage-side, clinical, FOB, hand-held and open-field observations, body weights, hindlimb grip performance, forelimb grip performance, rectal temperature or landing foot splay, at any dose. No effects were detected in any aspect of motor activity. An apparent increase in level of activity on a ranking basis on day 8 in males at 632 mg/kg bw per day was not confirmed by motor activity scores, and a slight variation in rectal temperature at 200 mg/kg bw per day was regarded as incidental as the findings were within normal variation within the study and there was no dose–response relationship (Table 14).

Neuropathology evaluation revealed some neural lesions, including focal/multifocal, very slight degeneration of individual nerve fibres in the trapezoid body of the medulla oblongata, the sciatic nerve, or the lumbar dorsal root ganglion, very slight mineralization of the nasal olfactory epithelium, and an epidermal inclusion cyst in the meninges of the lumbar spinal cord. These alterations were either found in a single rat in the control group or a single rat at the highest dose, or were distributed within groups in a similar incidence (Table 14).

The NOAEL was 2000 mg/kg bw per day, the highest dose tested (Shankar & Stebbins, 1999).

Table 14. Findings in a study of acute neurotoxicity in rats given quinoxyfen by gavage

Finding	Dose (mg/kg bw per day)			
	0	200	632	2000
Activity level, day 8, M/F (ranking)	2.2 / 3.1	2.5 / 3.3	2.7* / 3.4	2.5 / 3.3
Motor activity scores, day 8, M/F	10.6 / 11.4	9.9 / 13.0	9.4 / 12.6	10.7 / 11.9
Rectal temperature, day 8, M/F (°C)	36.5 / 37.3	36.9* / 37.3	36.7 / 37.3	36.7 / 37.3
Medulla oblongata slight degeneration, M/F (%)	40 / 80	NA	NA	60 / 60

Lumbar root ganglion slight degeneration, M/F (%)	0 / 0	NA	NA	20 / 0
Sciatic nerve slight degeneration, M/F (%)	20 / 0	NA	NA	0 / 0
Lumbar spinal cord, cyst, M/F (%)	20 / 0	NA	NA	0 / 0

From Shankar & Stebbins (1999)

F, female; M, male; NA, not analysed.

* $p < 0.05$

Chronic neurotoxicity was assessed as part of the GLP-compliant, long-term study of toxicity/oncogenicity with quinoxyfen (Redmond et al., 1995). Groups of 15 male and 15 female rats were randomly designated at the beginning of the study as a satellite group for assessment of toxicity and neurotoxicity at approximately 1 year after exposure to diets providing quinoxyfen at nominal doses of 0, 5, 20 or 80 mg/kg bw per day. Groups of 10 males and 10 females were included in the FOB and motor activity assays of the study of neurotoxicity. Groups of five males and five females rats were assigned to the neuropathology portion. FOB and motor activity assessments were performed before dosing and at 3, 6, 9 and 12 months. Neuropathology examinations were performed on perfusion-fixed tissue of the brain (nine sections) and peripheral nerves from rats in the control group and at the highest dose.

Quinoxyfen had no effect at any time on hand-held or open-field observations, grip performance or landing foot splay, either in males or in females. Neither did quinoxyfen affect any aspect of motor activity, either in males or in females. Occasional variations between test and control groups were not considered to be treatment-related as they were not consistent over time and/or were also evident in the pre-dosing phase. The main effect in this study was on body-weight gains. Although body weights revealed no treatment-related effect when statistically analysed, body-weight gains were affected in females at the highest dose (80 mg/kg per day). At 3 months, this group of rats had gained 10% less body weight than the females in the control group compared with their respective pre-exposure body weights. Also, at 12 months, the females at the highest dose had gained 11% less body weight than the females in the control group. The results of the neuropathological evaluation did not indicate that quinoxyfen had any effect on the central and peripheral nervous system.

The NOAEL for neurotoxicity was 80 mg/kg bw per day, the highest dose tested. The NOAEL for systemic toxicity was 20 mg/kg bw per day on the basis of reduced body-weight gain, which is consistent with the main segment of the long-term study in rats (Shankar et al., 1995).

3. Studies on metabolites

The acute oral toxicity of a compound initially identified as 3-hydroxy-quinoxfen (purity not stated) but subsequently considered to be 2-oxo-quinoxyfen (Pearson & Reeves, 2005) was investigated in a GLP-compliant study in female Fischer F344 rats. There were no deaths at the only dose tested, 5000 mg/kg bw (Merkel, 2004).

4. Observations in humans

A review of clinical and health surveillance data for the campaign periods of quinoxyfen reported no significant health effects related to potential exposure at the workplace.

One of the formulation chemists at the DowElanco research site at Letcombe Regis, was shown to be sensitized to aqueous solutions containing 10% or more quinoxyfen. No other cases had been found after checking all employees who have worked with the material. In addition, 20 control individuals

were patch-tested specifically with quinoxyfen and this produced an entirely negative reaction. The data from the formulation laboratory were suggested by the applicant to indicate that there is a small possibility for idiosyncratic skin sensitization. Since the formulation chemist may have been sufficiently exposed to be sensitized compared with other workers and given the additional evidence from animal experiments, this finding is consistent with quinoxyfen being a skin sensitizer (Foulds, 1994).

None of 28 individuals in a health surveillance programme reported or presented for health concerns related to quinoxyfen between June 1992 and March 1995. Audits of clinical records of all individuals involved in the investigations were performed and no health-related problems relating to quinoxyfen were noted (Burns, 1995).

An updated report contained an additional incident, where two operators and a supervisor were exposed to quinoxyfen while decanting a returned batch of a 50% w/w soluble concentrate formulation. All three developed rashes of the wrist/forearm. The supervisor and one operator were tested for sensitization to quinoxyfen, with positive results. The other operator had left the company before the follow-up testing was performed (Charles, Bass & Brownson, 2006).

Comments

Biochemical aspects

Phenyl- and quinoline-ring ^{14}C-labelled material was used to investigate the absorption, distribution, metabolism and excretion of quinoxyfen in rats. Differences in findings for each type of radiolabel were due to extensive cleavage of the ether bond. Absorption was rapid, with peak plasma concentrations of radioactivity (2–3 μg equivalents/g) seen within 1 h at a dose of 10 mg/kg bw. There were no data on tissue concentrations of radioactivity corresponding to the plasma C_{max}. Excretion of the quinoline radiolabel was relatively rapid (68–85% in 24 h) and predominantly in the faeces (approximately 70%) after absorption and subsequent secretion in the bile (approximately 50%). With the phenyl label, there was no marked difference between the proportion found in the urine or in the faeces. After most of the dose had been excreted (48 h), the highest concentrations of quinoline radiolabel were found in perirenal fat (0.12–0.35%/g), ovaries (0.07%/g), liver (0.03–0.05%/g), and kidneys (0.01–0.03%/g) with levels generally higher in females than in males. At 72 h after administration, the longest duration investigated, 1–2% of the radioactivity remained in the carcass and tissues. Metabolism involved cleavage of the ether linkage to give 4-fluorophenol and 5,7- dichloro-4-hydroxy quinoline and conjugation (unidentified) of both fluorophenyl and quinoline moieties; there was also direct conjugation to the fluorophenyl ring of intact quinoxyfen. There were no significant differences either between the sexes or between single or repeated administration at 10 mg/kg bw. Saturation of absorption and metabolism was evident at 500 mg/kg bw.

Toxicological data

Quinoxyfen was of low acute toxicity when administered orally (LD_{50} > 5000 mg/kg bw in rats), dermally (LD_{50} > 2000 mg/kg bw in rabbits) or by inhalation (LC_{50} > 3.4 mg/l in rats). Quinoxyfen was not irritating to skin, was slightly irritating to the eyes and was found to be a skin sensitizer using the maximization assay, but not when the Buehler method was used.

In short-term studies of toxicity with quinoxyfen, changes in liver weight and pathology and body-weight gain were the most consistently identified effects. In mice, increased liver weight, slight to moderate hepatocyte hypertrophy and very slight hepatocellular necrosis were present after 13 weeks of treatment at 500 mg/kg bw per day. The NOAEL for short-term toxicity in mice was 100 mg/kg bw per day. In rats, increased liver weight, and slight hepatocyte hypertrophy with increased basophilia were produced by doses of 100 mg/kg bw per day or above, with very slight hepatocellular necrosis

being observed at 250 mg/kg bw per day. The hepatic effects seen in the rat after 13 weeks at 100 mg/kg bw per day were reversible within 4 weeks, but some effects persisted at 250 mg/kg bw per day. In dogs, slight vacuolation of hepatocytes—considered by the investigators to be suggestive of dilated endoplasmic reticulum rather than lipid accumulation—was observed after 4 weeks of treatment at 250, 500 and 1000 mg/kg bw per day, with hepatocellular necrosis in females at 500 and 1000 mg/kg bw per day. In the 13-week study in dogs, slight hepatocellular hypertrophy was seen in one male at 100 mg/kg bw per day, the highest dose used in the study. In the 1-year study in dogs, increased hepatocyte size and serum ALP activity (two- to threefold) was seen in both sexes at 200 mg/kg bw per day.

Other findings in rats included reductions in body-weight gain and food consumption of males and/or females after 4 or 13 weeks of treatment at doses of ≥ 100 mg/kg bw per day. Kidney weight was increased at 250 mg/kg bw per day after 13 weeks of treatment. The NOAEL was 10 mg/kg bw per day on the basis of reduced body-weight gain at 100 mg/kg bw per day; however, it was noted that the NOAEL in the 2-year study in rats was 20 mg/kg bw per day. In dogs, reduced body weight or body-weight gain and reduced food consumption were seen in males and/or females after treatment at doses of greater than 100 mg/kg bw per day; but not after 13 weeks of treatment at doses of up to and including 100 mg/kg bw per day. Effects consistent with haemolytic anaemia and compensatory response (haematopoiesis in the spleen and liver) were manifest at 200 mg/kg bw per day in the 1-year study in dogs, and the NOAEL was 20 mg/kg bw per day.

In a 28-day study of dermal exposure, rats were given quinoxyfen for 5 days per week at doses of up to 1000 mg/kg bw per day (equal to 714 mg/kg bw per day averaged over the study). There were no findings of systemic or local toxicity. The NOAEL was 714 mg/kg bw per day.

In an 80-week study, mice received diets containing quinoxyfen at variable concentrations to give mean intakes of 0, 20, 80 or 250 mg/kg bw per day. There were no treatment-related changes in survival, or incidences of tumours or non-neoplastic lesions. The only significant findings were at the highest dose: reductions in body-weight gain in both sexes and increased relative kidney and liver weights in females. There was no evidence for carcinogenicity with quinoxyfen in this study. The NOAEL was 80 mg/kg bw per day.

In a 104-week study, rats received diets containing quinoxyfen at variable concentrations to given mean intakes of 0, 5, 20 or 80 mg/kg bw per day. There were no treatment-related changes in survival or tumour incidences. The only significant findings were at the highest dose: an increase in liver weight at 20 mg/kg bw per day after 24 months was not considered to be adverse as there was no dose-related response and no associated histopathology findings. Clinical chemistry changes included increased concentration of blood urea nitrogen in males and increased concentration of cholesterol in females. Relative liver and kidney weights were increased in both sexes at 24 months, although hepatocyte hypertrophy was increased in males at 12 months it was not seen after 24 months. The incidence of glomerulonephropathy was increased in males at 24 months. Quinoxyfen was not carcinogenic in this study. The NOAEL was 20 mg/kg bw per day, which was also the overall NOAEL in rats.

Quinoxyfen produced negative results in an adequate range of studies of genotoxicity in vitro and in vivo. The Meeting concluded that quinoxyfen was unlikely to be genotoxic.

On the basis of the absence of carcinogenicity in rodents and the absence of genotoxicity, the Meeting concluded that quinoxyfen is unlikely to pose a carcinogenic risk to humans

In a two-generation study of reproductive toxicity, there were no treatment-related differences in reproductive indices or in pup survival at doses of up to 110 mg/kg bw per day, the highest dose tested. The NOAEL for toxicity in parents and offspring was 20 mg/kg bw per day on the basis of a slight but consistent reduction in body-weight gain in pups during lactation and hepatocyte hypertrophy in parental males, in the absence of clinical chemistry examinations of serum enzyme activities.

In studies of developmental toxicity in rats, the NOAEL for maternal toxicity, fetotoxicity and teratogenicity was 1000 mg/kg bw per day, the limit dose. In the study of developmental toxicity in rabbits, the NOAEL for maternal toxicity was 80 mg/kg bw per day on the basis of a reduction in food consumption, reductions in maternal body-weight gain, poor condition and an increased incidence of abortions at 200 mg/kg bw per day. The abortions were noted to occur after 10 or more doses and in the presence of overt maternal toxicity, and were therefore not considered to be related to direct fetal toxicity. Quinoxyfen was not fetotoxic or teratogenic in rabbits under the conditions of this study.

Investigative studies were conducted on liver enzyme induction and hepatic effects in rats and mice treated with quinoxyfen for 2 weeks. In mice, significant increases in the activities of monoxygenase enzymes occurred at 1500, 6000 or 12 000 ppm. Hepatocellular hypertrophy was reported at 6000 ppm (equivalent to 890 mg/kg bw per day) and above. The NOAEL for liver enzyme effects in mice was 400 ppm (equivalent to 55 mg/kg bw per day). In rats, only *p*-nitroanisole *O*-demethylase activity was investigated; this enzyme showed increased activity at doses of 2500 ppm or above for 2 weeks. The NOAEL was 500 ppm (equal to 45 mg/kg bw per day) for both sexes. There were no treatment-related histopathological changes in the liver of rats exposed for 2 weeks at doses of up to 12 500 ppm, equal to approximately 1700 mg/kg bw per day, a dose producing significant mortality. The NOAELs for xenobiotic enzyme induction after 2 weeks exposure to quinoxyfen are above the NOAELs in the studies used to derive the acceptable daily intake (ADI).

In a study of acute neurotoxicity in rats, quinoxyfen exhibited no systemic toxicity or evidence of neurotoxicity at 2000 mg/kg bw. In an investigation conducted as part of the long-term study of toxicity in rats, the administration of quinoxyfen over 12 months did not produce any effect on motor activity or functional observations, nor on histopathology of the peripheral or central nervous system.

Workplace-monitoring data had confirmed three cases of skin sensitization associated with the handling of quinoxyfen in the initial phases of development and manufacture. No other adverse findings had been associated with the production, formulation or use of quinoxyfen.

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

Toxicological evaluation

The Meeting established an ADI for quinoxyfen of 0–0.2 mg/kg bw on the basis of NOAELs of 20 mg/kg bw per day identified in three studies: the 24-month study in rats, on the basis of reduced body-weight gain, liver and kidney effects at 80 mg/kg bw per day; the 1-year study in dogs, on the basis of reduced food consumption and body-weight gain, haematological effects, and liver effects at 200 mg/kg bw per day; and the two-generation study of reproductive toxicity in rats, on the basis of a reduction in body-weight gain in pups at 110 mg/kg bw per day during lactation; and with the application of a 100-fold safety factor.

The Meeting noted that quinoxyfen was not acutely toxic after short-term dosing, that there were no adverse findings in a study of acute neurotoxicity and that quinoxyfen did not exhibit specific developmental toxicity. The Meeting concluded that the establishment of an ARfD was unnecessary.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month study[c]	Toxicity	80 mg/kg bw per day	250 mg/kg bw per day
		Carcinogenicity	250 mg/kg bw per day[a]	—
Rat	Acute neurotoxicity[b]	Toxicity	2000 mg/kg bw per day[a]	—
	Two-generation study of reproductive toxicity[c]	Reproduction	110 mg/kg bw per day[a]	—
		Offspring	20 mg/kg bw per day	110 mg/kg bw per day
		Parental	20 mg/kg bw per day	110 mg/kg bw per day
	Developmental toxicity[b]	Maternal	1000 mg/kg bw per day[a]	—
		Developmental	1000 mg/kg bw per day[a]	—
	Two-year study[c]	Carcinogenicity	80 mg/kg bw per day[a]	—
		Toxicity	20 mg/kg bw per day	80 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal	80 mg/kg bw per day	200 mg/kg bw per day
		Developmental	80 mg/kg bw per day	200 mg/kg bw per day
Dog	One-year study[c]	Toxicity	20 mg/kg bw per day	200 mg/kg bw per day

[a] Highest dose tested.

[b] Gavage administration.

[c] Dietary study, variable concentrations used to give constant dose in mg/kg bw per day.

Estimate of acceptable daily intake for humans

0–0.2 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 exposures

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of absorption	Rapid and moderately extensive; about 70% of a dose of 10 mg/kg bw in rats, lower at higher doses.
Distribution	No data corresponding to C_{max}. Highest residual radioactivity in perirenal fat, skin, ovaries, liver, and kidneys
Potential for accumulation	Equivocal: 1–2% remaining at 72 h; log K_{ow} > 4
Rate and extent of excretion	Relatively rapid. For quinoline-ring label quinoxyfen in rats at 2 days: 13–20% in urine; 68–78% in faeces; for phenol label approximately 40% in urine and faeces
Metabolism in animals	Extensive hydrolytic cleavage to form 4-fluorophenol and 5,7-dichloro-4-hydroxyquinoline with subsequent conjugation. Some conjugation of uncleaved quinoxyfen.
Toxicologically significant compounds in animals, plants and the environment	Parent
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rabbit, LD_{50}, dermal	> 2000 mg/kg bw

Rat, LC_{50}, inhalation	> 3.4 mg/l of air (maximum achievable concentration)
Rabbit, skin irritation	Not irritating
Rabbit, eye irritation	Slight irritant
Guinea-pig, skin sensitization (test method used and result)	Positive in maximization test, negative in Buehler test
Short-term studies of toxicity	
Target/critical effect	Food consumption/body-weight gain; liver (rats, mice, dogs); haematological effects (dogs)
Lowest relevant oral NOAEL	20 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	714 mg/kg bw per day (28-day, 20 exposures, rats)
Genotoxicity	Negative in a battery of tests in vitro and in vivo. No genotoxic potential.
Long-term studies of toxicity and carcinogenicity	
Target/critical effect (rats)	Kidney & liver weight (both sexes) and glomerulonephrosis (males); body weight.
Lowest relevant NOAEL/NOEL	20 mg/kg bw per day (2-year study in rats)
Carcinogenicity	No evidence of treatment related tumorigenicity in rats or mice
Reproductive toxicity	
Reproduction target/critical effect	No effects on reproductive indices Reduction in body-weight gain in offspring during lactation
Lowest relevant reproductive NOAEL	20 mg/kg bw per day (parents and offspring) 110 mg/kg bw per day (reproduction)
Developmental target/critical effect	No specific fetal effects
Lowest relevant developmental NOAEL	80 mg/kg bw per day (maternal toxicity in rabbits). 80 mg/kg bw per day (fetal effects)
Neurotoxicity / delayed neurotoxicity	
Acute neurotoxicity	No adverse effects
12-month investigation of neurotoxicity within a long-term study in rats	No neurotoxic effects.
Other toxicological studies	
Induction of liver xenobiotic metabolizing enzymes (2-week dietary study)	NOAELs: 45 mg/kg bw per day (rats); 55 mg/kg bw per day (mice)
Medical data	
	Sensitization in production-plant personnel. No other adverse effects reported.

Summary

	Value	Study	Safety factor
ADI	0–0.2 mg/kg bw	Rat, 2-year study; dog, 1-year study; rat, study of reproductive toxicity	100
ARfD	Unnecessary	—	—

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TEMEPHOS

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Explanation

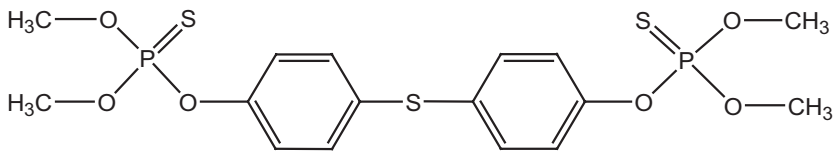
Temephos (*O,O,O'O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene bis(phosphorothioate)) is an organophosphate insecticide. Temephos is one of the compounds recommended by the World Health Organization (WHO) as a mosquito larvicide treatment for potable water. No acceptable daily intake (ADI) had been established previously. For that reason, the WHO Drinking-water Guidelines

programme recommended that temephos be evaluated toxicologically by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). Temephos has not been previously evaluated by the Meeting.

For temephos, the specifications were established by the Joint FAO/WHO Meeting on Pesticide Specifications (JMPS) and published as *WHO specifications and evaluations for public health pesticides*: *temephos* (WHO, 1999).[1]

Several studies with temephos, including studies of acute oral toxicity, dermal toxicity inhalation toxicity, irritancy, skin sensitization, genotoxicity and special studies of neurotoxicity in hens, were certified as being compliant with good laboratory practice (GLP). Other available studies were carried out before the Organisation for Economic Co-operation and Development (OECD) guidelines on GLP were promulgated. The Meeting noted that the reports of some of the critical studies were available only as brief published articles that lacked some of the details normally required for completing an adequate risk assessment.

Figure 1. Structural formula of temephos



The specifications for technical temephos (WHO/SIT/19.R4), temephos emulsifiable concentrate (WHO/SIF/31.R4), temephos emulsifiable concentrate for simulium control (WHO/SIF/34.R3) and temephos sand granules (WHO/SIF/40.R1) were last revised on 10 December 1999.

Temephos is a phenyl organothiophosphate insecticide that is mainly used as a larvicide to control mosquitoes, midge, blackfly and other insects. Its toxic action against the target species is by inhibition of cholinesterase in the nervous system. Temephos has been used for the control of mosquito larvae in potable water since the early 1970s (WHO, 1991) and has been recommended for treatment of potable water at a dose not exceeding 1 mg/l (WHO, 1984; Rozendaal, 1997).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, excretion and biotransformation

Rats

Groups (number and sex not stated) of Sprague-Dawley rats (body weight, 150–200 g) were given radiolabelled temephos at a dose of 970, 1357, 3164 or 9168 µg (dissolved in acetone and sesame oil) given by oral gavage. The temephos was radiolabelled with tritium at the ortho-position relative to the sulfide linkage on both phenyl rings of the molecule. Samples of urine and faeces were collected for analysis at various times between 7 h and 96 h after dosing. Unspecified numbers of animals from the group at 970 µg were killed at 48 h and rats from the group at 1357 µg were killed at 72 h. Samples of muscle, liver, kidney, stomach, intestines and fat were taken for analysis. The urine for each group at each time was pooled and extracted with chloroform and ethyl ether before analysis

[1] Available from: http://www.who.int/whopes/quality/en/temephos.pdf

by thin-layer chromatography (TLC). Faecal samples were extracted with methanol and analysed by TLC. Residues in fat were extracted with hexane and methanol and analysed by TLC. Identification of metabolites was by chromatographic comparisons with non-radioactive reference compounds. Tissues other than fat contained insufficient residues to permit analysis by TLC. The measurement of amounts of radiolabel in different tissues was by liquid scintillation counting.

More than 95% of the administered radioactivity was recovered in faeces and urine within 98 h, with most being recovered in the first 48 h. The distribution of radiolabel in the various tissues is shown in Table 1, and shows that most of the residues were in the fat.

Table 1. The tissue distribution of radiolabel in rats given tritiated temephos

Tissue	Concentration of radiolabel (µg temephos equivalents per gram tissue)	
	970 µg/rat at 48 h	1357 µg/rat at 72 h
Fat	1.25	1.75
Intestines	NA	0.51
Kidneys	0.07	0.09
Liver	0.17	0.19
Muscle	0.03	0.02
Stomach	0.04	0.24

From Blinn (1966)
NA, no data available.

The metabolites of temephos that were identified were temephos sulfoxide, 4,4'thiodiphenol, 4,4'-sulfinyldiphenol and 4,4'-sulfonyldiphenol. Faeces contained 60% of the total radioactivity recovered, with 30% as temephos, 10% as its sulfoxide, 5% as 4,4'-thiodiphenol, 4% as 4,4'-sulfonyldiphenol and 10.5% as unknowns and unextractables. Urine samples contained 39.5% of the total recovered radiolabel. The amounts of metabolites extractable (as percentages of the total radioactivity recovered from urine, faeces and tissues) from untreated urine were 0.5%, 2%, 1.5% and 2.2% for temephos, 4,4'-thiodiphenol, 4,4'-sulfinyldiphenol and 4,4'-sulfonyldiphenol. These amounts increased to 0.5%, 24%, 2.5% and 4.6%, respectively, when the urine was treated with a mixture of glucuronidases and sulfatases, indicating that some of the urinary metabolites (particularly the 4,4'-thiodiphenol) were present in conjugated form. Only about 0.5% of the total recovered radiolabel was found in samples of fat taken at 48 h and 72 h after dosing. Of this, 0.5% from fat tissue, 0.3% was as temephos and 0.2% as its sulfoxide. It was proposed that the metabolism of temephos can occur by *S*-oxidation to form temephos sulfoxide and by carboxylesterase hydrolysis to form 4,4'-thiodiphenol. Further hydrolysis and *S*-oxidation of these metabolites would result in the formation of 4,4'-sulfinyldiphenol and 4,4'-sulfonyldiphenol. Secondary metabolism by glucuronidation or sulfation of temephos, 4,4'-thiodiphenol, 4,4'-sulfinyldiphenol and 4,4'-sulfonyldiphenol would result in the formation of conjugates (Blinn, 1966).

2. Toxicological studies

2.1 Acute toxicity

(a) Oral toxicity

Mice and rats

Studies of acute oral toxicity were performed with CF-1 mice and CHRCD rats. Certificates of compliance with GLP were not provided with the reports of these studies

In a study in mice, groups of five females were given single doses of temephos at 1250, 2500 or 5000 mg/kg bw in corn oil by oral gavage. The treatment was followed by a 14-day observation period. Body weights were measured at the beginning and end of the study. Necropsies were not performed. All animals at 5000 mg/kg bw died before the end of the observation period. At all doses, ataxia, decreased activity, diarrhoea and prostration were observed. Salivation and diuresis were also observed at 1250 and 2500 mg/kg bw. The oral median lethal dose (LD_{50}) for female mice in this study was estimated to be 2062 mg/kg bw (Fischer, 1986; Nielsson, 1990).

In a study in rats, groups of five males and five females were given single doses of temephos at 2500, 5000 or 10 000 mg/kg bw in corn oil by oral gavage. The treatment was followed by a 14-day observation period, during which body weights were measured weekly. Autopsies were performed on all rats. All of the males at 10 000 mg/kg bw, but none of the females at this dose died during the observation period. Decreased movement was seen in all animals at all doses and diuresis was seen in all rats at 5000 mg/kg bw or more. At autopsy, all of males at the highest dose had discoloured liver and haemorrhagic or dark coloured lungs and three out of five had blood in the lumen of their intestines. No adverse findings were seen in females at the highest dose. The oral LD_{50} was 4204 mg/kg bw in males and > 10 000 mg/kg bw in females (Fischer, 1986; Nielsson, 1990).

In a briefly reported study that did not conform with GLP, groups of 20–58 male and 20–58 female adult Sherman rats were given single doses of three different batches of technical-grade temephos (purity not reported) in undiluted form in various volumes by oral gavage. Similarly, a group of 50 adult female white mice (strain not reported) were treated orally with various volumes of temephos from one of the batches (batch A). In addition, groups of 20 male and 20 female rats received temephos of batch A in various volumes applied to the skin and were restrained to stop them temephos being removed by licking. The animals were observed for 22 days. The lowest oral dose that caused death in rats was 4000 mg/kg bw. The affected animals were reported to show "the classical symptoms of illness and manner of death associated with organic phosphorus poisoning". The acute oral toxicity of batch A appeared to be lower than that of batches B and C in rats, and was also higher in mice than in rats (Table 2) (Gaines et al., 1967).

Guinea-pigs

In another briefly reported experiment that did not conform with GLP, a group of five adult male guinea-pigs was given temephos (purity not stated) as five daily doses at 100 mg/kg bw administered by gavage. The animals showed no clinical signs of toxicity and findings on autopsy were unremarkable. Cholinesterase activities were not measured (Gaines et al., 1967).

Rabbits

In a briefly-reported study that did not comply with GLP, a group of eight rabbits was given temephos at an oral dose at 100 mg/kg bw per day for 5 days, while a control group of six rabbits was not treated. Three treated rabbits died during the dosing period. Autopsies were performed on seven of the treated rabbits and all control animals. No microscopic examination of tissues was conducted. Diffuse necrosis of the liver was reported in two treated rabbits and focal liver necrosis was reported in another two treated rabbits, while no liver necrosis was found in untreated controls (Gaines et al., 1967).

Birds

In a study that complied with GLP, groups of four mature White Leghorn chickens (sex not stated) were given temephos as single oral doses at 215, 464, 681, 1000 or 1470 mg/kg bw by gavage. An acute oral LD_{50} of 500 mg/kg bw was calculated from the mortality results (Fletcher & Leonard, 1986).

In a special study to investigate the potential of temephos to cause neurotoxicity, groups of six adult hens (breed not stated) were given temephos (purity, 99.9%) as single oral doses of 0 (untreated controls), 227, 455, 823, 1137, 1422 or 1705 mg/kg bw by gavage. Mortality in the group at 227 mg/kg bw was no greater than in the untreated control group. The oral LD_{50} calculated from the results for mortality in this study was 579 mg/kg bw (Ross et al., 1976). More details of this study are given in the section "Neurotoxicity studies in hens".

Groups of three adult White Leghorn hens were given various amounts of temephos (purity, 87.1%) as single oral doses. From the mortality results, an oral LD_{50} of 183 mg/kg bw was calculated (McNerney & Levinskas, 1967).

Groups of 2–11 hens were given single oral doses of temephos of three different batches (A, B and C) (purity not stated) at 125, 250, 500 or 1000 mg/kg bw by gavage. All doses caused leg weakness, but the effect was reported as being very mild in the group at 125 mg/kg bw and disappeared within 6 days. The leg weakness lasted longer in the other groups. Half of the animals (5 out of 10) died in the group at 1000 mg/kg bw. No difference in response was detected between groups given the same doses of different batches of temephos (Gaines et al., 1967).

Groups of 5–8 chicks or ducklings were given temephos (purity not stated) as five daily oral doses at 10, 25 or 50 mg/kg bw by gavage. All of the birds at 50 mg/kg bw per day died, as did several of those at 25 mg/kg bw per day (two out of six chicks and five out of eight ducklings). The survivors in the group at 25 mg/kg bw per day were generally weak, but typical leg weakness was not observed. The six chicks at 10 mg/kg bw per day showed no clinical signs at all, but four of the five ducklings at this dose showed mild signs of poisoning over the first 2 days, but had recovered by the end of the dosing period. Autopsy findings were unremarkable (Gaines et al., 1967).

Groups of 10 hens were given temephos (purity not stated) as a single subcutaneous dose at 500 or 1000 mg/kg bw. Five of the hens given 1000 mg/kg bw died and all of the survivors developed leg weakness which persisted for 6 to 38 days (mean, 26 days). All of the hens given 500 mg/kg bw survived, but they all developed leg weakness that persisted for 11 to 31 days (mean, 15 days). The onset of leg weakness was immediate but recovery was gradual (Gaines et al., 1967).

(b) Dermal toxicity

Rabbits

In a briefly-reported study for which a GLP certificate was not supplied, temephos (purity, 94.7%) was applied as single doses at 1000, 2000 or 4000 mg/kg bw to the intact shaved skin of groups of five male and five female New Zealand White rabbits. The application site was covered with occlusive dressing for 24 h, and was then wiped with moistened gauze. The animals were observed for 14 days after treatment and body weights were measured weekly. All animals were killed for autopsy at the end of the observation period. Decreased activity and body-weight loss were reported at all doses and those rabbits at 200 mg/kg bw or more also had diarrhoea, prostration and anorexia. Autopsies showed gross pathology in the animals at the highest dose: blood in the abdominal cavity (7 out of 10 rabbits), haemorrhagic areas in the intestines (8 out of 10), and pale kidneys (7 out of 10). The LD_{50} was estimated to be 2000 mg/kg bw in males and 2378 mg/kg bw in females (combined results for both sexes, 2181 mg/kg bw) (Fischer, 1986).

Table 2. Oral and dermal median lethal doses of temephos

Species	Sex	Route	Batch of temephos	LD_{50} (mg/kg bw)	Reference
Mouse	Male (50/group)	Oral	A	4 700	Gaines et al. (1967)
Mouse	Female (5/group)	Oral	—	2 062	Fischer (1986)
Rat	Male (58/group)	Oral	A	8 600	Gaines et al. (1967)
Rat	Female (50/group)	Oral	A	13 000	Gaines et al. (1967)
Rat	Female (20/group)	Oral	B	4 000	Gaines et al. (1967)
Rat	Female (20/group)	Oral	C	4 000	Gaines et al. (1967)
Rat	Male (5/group)	Oral	—	4 240	Fischer (1986); Nielsson (1990)
Rat	Female (5/group)	Oral	—	> 10 000	Fischer (1986); Nielsson (1990)
Chickens	Not stated (4/group)	Oral	—	500	Fletcher & Leonard (1986)
Chickens	Female (3/group)	Oral	—	183	McNerney & Levinskas (1967)
Rat	Male (20/group)	Dermal	A	> 4 000	Gaines et al. (1967)
Rat	Female (20/group)	Dermal	A	> 4 000	Gaines et al. (1967)
Rabbit	Male (5/group)	Dermal	—	2 000	Fischer (1986)
Rabbit	Female (5/group)	Dermal	—	2 378	Fischer (1986)

LD_{50}, median lethal dose

(c) Inhalation

Rats

The acute toxicity of temephos administered by inhalation was examined in a GLP-compliant study in Sprague-Dawley rats. Groups of 10 male and 10 females were exposed to temephos (purity, 94.7%) at measured concentrations of 3.49, 4.20, 4.37, 4.73 or 4.79 mg/l in an exposure chamber for 4 h. The mass median aerodynamic diameter (MMAD) of the particles to which the groups were exposed ranged from 1.80 to 1.93 µm. The rats were observed for 14 days after exposure. On day 15, all surviving rats were killed for autopsy. There was no significant difference in the findings for the different groups. Body weights increased in all groups over the course of the study. Clinical signs included ruffled fur, hypersensitivity and slight tremors. The clinical signs generally appeared to be more severe in males than in females. Mortality varied between 0% and 30%. In decedent animals, autopsy findings included small spleen and haemorrhages in the thymus, lung and around the kidneys. In animals surviving to the end of the study, the most common finding was congested lungs. It was

concluded that temephos was of low acute inhalation toxicity with a median lethal concentration (LC_{50}) of > 4.79 mg/l (Hershman, 1986).

(d) Dermal irritation

Guinea-pigs

Male Hartley guinea-pigs were given temephos (purity, 94.7%) in a test for dermal irritancy as a screen before a test for dermal sensitization. Groups of one animal received temephos at concentrations of 25, 50, 75 and 100% (w/v) applied to the clipped skin for a contact period of 6 h. When the application sites were examined after 24 h and 48 h, there was no erythema or oedema at any of the application sites. In was concluded that temephos was not irritant to the skin of guinea-pigs (Costello, 1986).

Rabbits

The dermal irritation potential of temephos was investigated in six New Zealand White rabbits. Undiluted temephos (purity, 94.7%) was applied to shaved and to shaved and abraded areas of skin on the flanks of each rabbit. The test material was left for 24 h, after which the application sites were cleaned with moistened gauze. The application sites were examined at the end of the application period and at 72 h after dosing. No signs of irritation were seen at any of the abraded or unabraded sites on any animal at either examination time. It was concluded that temephos was not a skin irritant in this test (Fischer, 1986).

(e) Ocular irritation

Rabbits

The ocular irritation potential of temephos was investigated in six New Zealand White rabbits. Undiluted temephos (purity, 94.7%) was applied to the conjunctival sac of the right eye of each rabbit. The left eye was left untreated to act as a control. The test material was left for 24 h, after which the eyes were rinsed with tap water and examined for irritation. Further examinations were performed at 1, 24, 48 and 72 h after dosing. No signs of eye irritation were seen in any animal at any examination time. It was concluded that temephos was not an eye irritant in this test (Fischer, 1986).

(f) Dermal sensitization

In a modified Buehler test, guinea-pigs were allocated to a test group, a negative-control group or a positive-control group. Each group consisted of 11 animals. For the test group, 0.42 ml of undiluted temephos (purity, 94.7%) was applied to a gauze patch, which was placed on the clipped skin of each animal. The trunk of the animal was then wrapped in plastic and secured with an elastic bandage. After a contact period of 6 h, the patch was removed. The site of application was examined at 24 h and 48 h. This sequence was repeated three times per week during an induction phase, until nine applications had been made. After a rest period of 2 weeks, a challenge application of temephos was made to the clipped skin and left for 6 h, followed by observations at 24 h and 48 h. In the positive-control group, a suspension of 1-chloro-2,4-dinitrobenzene was used instead of temephos. In the negative-control group, the animals remained untreated during the induction stage and only received the challenge application of temephos. No erythema or oedema was seen in any animal in the test group or the negative-control group during the induction phase or after challenge. Ten out of eleven animals in the positive-control group showed erythema and/or oedema at the challenge application site. It was concluded that temephos showed no skin sensitizing potential in this test (Costello, 1986).

2.2 Short-term studies of toxicity

(a) Oral toxicity

Short-term studies of oral toxicity after repeated doses have been performed in rats and dogs given diets containing temephos. None of the studies were performed to the standards of GLP as they were all conducted before the introduction of GLP standards.

Rats

Groups of 45 male and 45 female Carworth Farms' Nelson strain rats were given diets containing temephos (purity, 96.4%) at a concentration of 2, 6, 18 or 350 ppm for 92 days. These dietary concentrations supplied doses of approximately 0.15, 0.46, 1.41 and 32 mg/kg bw per day, to males and 0.18, 0.54, 1.71 and 35.2 mg/kg bw per day to females. An untreated control group consisted of 65 males and 65 females. A non-standard protocol was used. Food consumption and body weight were measured weekly for 10 males and 10 females in each group. Rats were randomly chosen from the different groups for determination of cholinesterase activity in plasma, erythrocytes and brain. Seven rats of each sex from the control group and four rats of each sex from the groups at 2, 6 and 18 ppm were killed for testing after 1, 3, 5, 9 and 13 weeks, and four rats of each sex at 350 ppm were killed for cholinesterase estimation after 12 weeks. After 13 weeks, 10 rats of each sex selected from the groups at 2, 6 and 18 ppm, and 15 rats of each sex from the control groups were fed control diet for a further 2–4 weeks before testing for cholinesterase activity in plasma, erythrocytes and brain. All animals at 350 ppm were autopsied after 12 weeks of dosing. After 13 weeks, 15 rats of each sex from the remaining groups (including controls) were autopsied. Liver and kidney weights were recorded and a wide selection of tissues was preserved for microscopic examination.

The results showed no effect on feed intake, and body-weight gain was reduced only in females at the highest dose of 350 ppm. There was no treatment-related effect on mortality. At 350 ppm, there was marked inhibition of cholinesterase activity in plasma, erythrocytes and brain in both sexes. At 18 ppm, cholinesterase activity was inhibited in erythrocytes and brain at most time-points throughout the study, with inhibition of erythrocyte cholinesterase activity being greater than 20% after 5 weeks in males and after 3 weeks in females. At 6 ppm, only erythrocyte cholinesterase was inhibited: in males at weeks 9 and 13, and in females at week 13 only. Inhibition in males was less than 20% and was not regarded as toxicologically significant. The 27% inhibition in females at 6 ppm at 12/13 weeks was regarded as an isolated finding and it was considered that there was no consistent adverse effect at this dose. At 2 ppm, there was slight inhibition (9%) of erythrocyte cholinesterase activity only at week 13 in females. After a 4-week recovery period, the cholinesterase activities in the treated groups were no different from those of the controls. The results for effects on erythrocyte cholinesterase activity are summarized in Table 3. Liver weights were statistically significantly lower than control values in males at 2 ppm and 350 ppm and in females at 18 ppm. Kidney weights were unaffected. Microscopic examination of tissues from rats at 350 ppm showed no treatment-related pathology. Tissues from groups given lower doses were not examined microscopically. Less than 20% inhibition of acetylcholinesterase activity was not regarded as being toxicologically significant. The no-observed-adverse-effect level (NOAEL) for the study was 6 ppm (1.41 mg/kg bw per day in males and 1.71 mg/kg bw per day in females) on the basis of consistent inhibition of erythrocyte acetylcholinesterase activity by 20% or more (Levinskas & Shaffer, 1965).

Table 3. Effects on mean erythrocyte cholinesterase activity in rats fed diets containing temephos

Dietary concentration (ppm)	Corrected ΔpH/h per 0.04 ml packed erythrocytes (percentage inhibition compared with value for concurrent control)						
	Treatment period					Recovery period	
	Week 1	Week 3	Week 5	Week 9	Week 12/13	Week +2	Week +4
Males							
0	0.494	0.473	0.446	0.450	0.507	0.579	0.519
2	0.468	0.445	0.458	0.433	0.458	0.565	0.542
6	0.505	0.452	0.442	0.378* (16%)	0.423* (17%)	0.548	0.505
18	0.422* (15%)	0.385* (19%)	0.342* (23%)	0.343* (24%)	0.323* (36%)	0.470* (19%)	0.475
350	—	—	—	—	0.040* (92%)	—	—
Females							
0	0.484	0.501	0.396	0.471	0.666	—	0.569
2	0.473	0.475	0.435	0.438	0.540* (9%)	—	0.562
6	0.468	0.440	0.365	0.433	0.483* (27%)	—	0.542
18	0.430	0.382* (24%)	0.338	0.375* (20%)	0.435* (35%)	—	0.515
350	—	—	—	—	—	—	—

From Levinskas & Shaffer (1965)

* $p < 0.05$ (differs significantly from control values)

In a second feeding study in rats, groups of 20 male and 20 female Carworth Farms' Nelson strain rats were given diets containing temephos (purity, 87.1%) at a concentration of 6, 18 or 54 ppm for up to 90 days (corresponding to doses of approximately 0.26, 0.78 and 2.35 mg/kg bw per day). A control group of 32 male and 32 female rats was given diets without temephos. Limited haematology (erythrocyte volume fraction, haemoglobin, total leukocyte count and differential leukocyte count) was performed on seven males and seven females sexing the control group and five males and five females from each treatment group before the start of treatment and after 11 (males) or 12 weeks (females). Samples were taken from seven males and seven females in the control group and five males and five females from each treatment group after 4, 8 and 13 weeks of treatment and after a recovery period of 2 weeks, for measurement of cholinesterase activity in plasma, erythrocytes and brain. No measurements were made of body weight, feed intake, organ weights, gross or microscopic pathology.

The only effect revealed by haematology was a slight increase in lymphocyte counts in males at 18 ppm, but this was not thought to be caused by the treatment. The results for cholinesterase activities in the erythrocytes and the brain are summarized in Tables 4 and 5. Both sets of results show a dose-related inhibition at dietary concentrations of 18 and 54 ppm at various times, and no statistically significant effect at 6 ppm. The 2-week recovery period was of insufficient duration to allow recovery of cholinesterase activities. For plasma cholinesterase, the dose–response relationship was less clear, with some occasions when cholinesterase was significantly inhibited at 6 ppm, so a

no-observed-effect level (NOEL) for inhibition of plasma cholinesterase activity could not be identified. Statistically significant inhibitions of erythrocyte and brain acetylcholinesterase were seen at 18 ppm, but inhibition was less than 20% (the cut-off for toxicological significance that is used by the Joint Meeting) in all cases but one (males at 4 weeks), which was regarded as an outlier result.

The NOAEL for consistent adverse effects on erythrocyte acetylcholinesterase activity was 18 ppm (0.78 mg/kg bw per day), while for brain acetylcholinesterase activity it was the highest dose tested, 54 ppm (2.35 mg/kg bw per day) (Hutchinson, et al., 1966).

Table 4. Effects on mean erythrocyte cholinesterase activity in rats fed diets containing temephos

Dietary concentration (ppm)	Corrected ΔpH/h per 0.04 ml packed erythrocytes (percentage inhibition compared with value for concurrent control)			
	Treatment period			Recovery period
	Week 4	Week 8	Week 13	Week +2
Males				
0	0.471	0.411	0.486	0.389
6	0.465	0.360	0.482	0.370
18	0.358* (24%)	0.340* (17%)	0.400* (18%)	0.405
54	0.248* (44%)	0.220* (46%)	0.220* (55%)	0.260* (33%)
Females				
0	0.404	0.476	0.511	0.493
6	0.418	0.420	0.510	0.478
18	0.350* (13%)	0.402* (16%)	0.458	0.480
54	0.290* (28%)	0.315* (34%)	0.395* (23%)	0.438

From Hutchinson et al. (1966)
* $p < 0.05$ (differs significantly from control values)

Table 5. Effects on mean brain cholinesterase activity in rats fed diets containing temephos

Dietary concentration (ppm)	Corrected ΔpH/h per 2.0 ml homogenate (percentage inhibition compared with value for concurrent control)			
	Treatment period			Recovery period
	Week 4	Week 8	Week 13	Week +2
Males				
0	1.239	1.336	1.229	1.191
6	1.222	1.290	1.205	1.150
18	1.092	1.190	1.078* (12%)	1.048* (12%)
54	1.242	1.105* (17%)	1.023* (18%)	1.015* (12%)

Females				
0	1.091	1.224	1.324	1.394
6	1.070	1.200	1.320	1.358
18	1.020	1.140	1.250	1.255* (10%)
54	1.015	1.048*	1.265 (10%)	1.242* (11%)

From Hutchinson et al. (1966)
* $p < 0.05$ (differs significantly from control values)

A series of experiments that did not comply with GLP, in which male Sherman-strain rats were given temephos of unstated purity in the diet or by oral gavage in peanut oil, were briefly reported.

In the experiment in which temephos was administered by oral gavage, groups of eight rats were given temephos at a dose of 0, 1, 10 or 100 mg/kg bw per day for up to 28 days, and groups of seven rats were given temephos at a daily oral dose of 0, 1, 10 or 100 mg/kg bw for up to 44 days. No adverse effects were reported for the group at 1 mg/kg bw per day. Rats receiving temephos at 10 mg/kg bw per day showed a 31% inhibition in erythrocyte cholinesterase activity after 14 days and a 47% inhibition after 44 days. None of these animals displayed signs of organophosphate poisoning. At 100 mg/kg bw per day, the rats showed signs that were reported to be "typical of organic phosphate poisoning" after three daily doses, at which time their erythrocyte cholinesterase activity was inhibited by 64%. As dosing progressed, the group at 100 mg/kg bw per day showed some recovery in the severity of their signs of toxicity, although inhibition of erythrocyte cholinesterase activity rose to 87% after 11 days of dosing. At this point in the experiment, dosing was stopped for an unspecified number of animals from this group and they were allowed a recovery period of 32 days. At the end of the recovery period, erythrocyte cholinesterase activity was inhibited by 27%, compared with controls. Findings from gross pathology and histopathology on rats from all groups showed no treatment-related effects. The NOAEL for this experiment was 1 mg/kg bw per day, with toxicologically significant inhibition (i.e. > 20%) of erythrocyte cholinesterase activity at 10 mg/kg bw per day or more.

In the dietary dosing experiment, groups of 10–14 rats were given diets containing temephos at a concentration of 0, 2, 20, 200 or 2000 ppm for 99 days. The doses received by the groups of rats over the course of the experiment were calculated to be approximately 0 mg/kg bw per day (untreated controls), 0.12 to 0.24 mg/kg bw per day (2 ppm), 1.2–2.4 mg/kg bw per day (20 ppm), 11–24 mg/kg bw per day (200 ppm) and 150 mg/kg bw per day (2000 ppm). All the rats in the group at 2000 ppm showed signs of organophosphate poisoning and 8 out of the 10 rats in the group died between days 5 and 10, having 100% inhibition of erythrocyte cholinesterase activity. The two remaining rats in this group survived until the end of the 99-day study, despite having a reported 100% and 80% inhibition of erythrocyte and plasma cholinesterase activities, respectively. Rats at 200 ppm or less did not show signs of cholinesterase poisoning. At 200 ppm, the rats showed a progressive inhibition of erythrocyte cholinesterase activity that was almost complete by day 14, but gradually reduced to 71% by day 99. No significant inhibition of cholinesterase activity (not stated whether this refers to erythrocyte or plasma activity or both) occurred at 2 or 20 ppm (equal to mean doses of 0.18 and 1.8 mg/kg bw per day respectively). Findings from gross pathology and histopathology on rats from all groups showed no treatment-related effects. The NOAEL was 20 ppm (equivalent to an average dose of 1.8 mg/kg bw per day or a range of doses of 1.2 to 2.4 mg/kg bw per day), with inhibition of erythrocyte cholinesterase activity at 200 ppm (equal to 11–24 mg/kg bw per day) or more.

The authors reported that temephos was slightly less toxic when given in the diet than when given by gavage. However, there was a greater degree of inhibition of erythrocyte cholinesterase activity (> 71%) seen at 200 ppm (11–24 mg/kg bw per day) in the dietary study than in the gavage

study (31–47%) at 10 mg/kg bw per day. The small differences in the NOAELs for the different ways of administering temephos orally can be explained by dose spacing and do not necessarily reflect a difference in toxicity (Gaines et al., 1967).

Rabbits

In one of a series of briefly reported experiments that did not comply with GLP, groups of four weanling male white rabbits (strain not stated) were given temephos (batch A; purity not stated) at a daily oral gavage doses of 0.1, 1.0 or 10 mg/kg bw for up to 35 days. It was not clear whether a control group had been used. None of the animals developed clinical signs of poisoning. After 7 days of treatment, the rabbits at 10 mg/kg bw per day showed a 26% inhibition of erythrocyte cholinesterase activity when compared with values for concurrent controls. At the end of 35 days, the degree of inhibition had risen to 47%. No treatment-related pathology was seen at autopsy. The NOAEL was 1.0 mg/kg bw per day on the basis of inhibition of erythrocyte cholinesterase activity at 10 mg/kg bw per day (Gaines et al., 1967).

In further experiments, groups of three to four rabbits were given temephos from one of three different batches (batches A, B and C, as fed to rats in a separate experiment described previously) at a daily oral dose of 10 mg/kg bw for 30 days by gavage, while groups of 4–12 rabbits were given a daily oral dose of 100 mg/kg bw per day by gavage for 5 days. The results for the different batches of temephos were similar. A dose of 10 mg/kg bw per day for 30 days caused "mild changes in liver pathology" in one out of three rabbits given temephos of batch A, one out of four given batch B and two out of three given batch C. While a dose of 100 mg/kg bw per day caused death in about one third of the animals (batch A, 4 out of 12; batch B, 1 out of 4; batch C, 1 out of 4) and in several animals caused focal necrosis (batch A, 6 out of 12; batch B, 1 out of 4; and batch C, 0 out of 4) or diffuse necrosis (batch A, 2 out of 12; batch B, 1 out of 4; and Batch C, 1 out of 4) of the liver. It was not clear whether the descriptions of liver changes were based on histopathological examination or solely on observations at autopsy. The lowest-observed-adverse-effect level (LOAEL) for this experiment was 10 mg/kg bw per day, with mild hepatotoxicity seen in some animals at this dose (Gaines et al., 1967).

Dogs

In a 90-day study, groups of beagle dogs were given diets containing temephos (purity, 96.4%) at a concentration of 0, 2, 6, 18 or 700/500 ppm (equivalent to approximately 0, 0.1, 0.3, 0.9 and 35/25 mg/kg bw per day). Each group consisted of two male and two female dogs, except for the group at 18 ppm which consisted of one male and three females. The highest dose was 700 ppm (17.5 mg/kg bw per day), which was given for the first 15 days, after which the dose was reduced to 500 ppm (12.5 mg/kg bw per day), until the end of week 12, when dogs receiving the highest dose were killed for necropsy. After 13 weeks of treatment with temephos at 0, 2, 6 or 18 ppm, the dogs were fed untreated diet for a 2-week recovery period. Dogs were weighed at approximately weekly intervals and physical examinations were carried out at 1–2 month intervals. Cholinesterase activity was measured in plasma and erythrocytes taken before treatment and after 1, 3, 5, 9 and 12/13 weeks of treatment (12 weeks for the group at the highest dose; 13 weeks for other groups) and at the end of the 2-week recovery period. Weights of liver, heart, kidneys and testes were measured in dogs in the group receiving the highest dose that underwent necropsy, brain cholinesterase activity was measured and a wide range of organs were examined microscopically.

No mortality occurred at any dose. The group at the highest dose showed cholinergic signs during the initial 2 weeks during which they were given temephos at 700 ppm, and showed lesser cholinergic signs during the rest of the study when they received temephos at the lower dietary concentration of 500 ppm. There was no treatment-related effect on body-weight gain. There was no significant inhibition of plasma or erythrocyte cholinesterase activities at dietary concentrations

of up to 18 ppm. In the group at the highest dose, there were inhibitions of plasma and erythrocyte cholinesterase (> 95%) at week 1 when they were fed temephos at 700 ppm and at subsequent weeks when they received temephos at 500 ppm. Marked inhibition of brain cholinesterase activity was also reported as occurring in the group at the highest dose, but control values were not given. Necropsies and microscopic examinations of the animals receiving the highest dose showed no treatment-related pathology. The NOAEL was 18 ppm, equivalent to 0.9 mg/kg bw per day, on the basis of inhibition of brain and erythrocyte activity at the highest dose, 700/500 ppm (35/25 mg/kg bw per day) (Hutchinson & Levinska, 1965).

In a briefly-reported study that did not comply with GLP, groups of one male and one female adult dogs (breed not stated) were given temephos (purity not stated) at a concentration of 10 or 50 ppm (equivalent to 0.6–0.8 or 3–4 mg/kg bw per day) for 129 days. None of the dogs showed clinical signs of toxicity. In the male given temephos at 50 ppm, erythrocyte cholinesterase activity fell to 67% of normal values (33% inhibition) in the first week and 22% of normal values (78% inhibition) by the end of the experiment. In the female given temephos at 50 ppm, erythrocyte cholinesterase activity remained normal until day 60, but fell to 50% of normal values (50% inhibition) on day 90 and remained similarly low until the end of the experiment. In the dogs at 10 ppm, erythrocyte cholinesterase activity was normal throughout the study. Plasma cholinesterase activity remained normal throughout the study in both groups. The NOAEL was 10 ppm, equal to 0.6–0.8 mg/kg bw per day, on the basis of inhibition of erythrocyte cholinesterase activity at 50 ppm, equal to 3–4 mg/kg bw per day (Gaines et al., 1967).

(b) Dermal toxicity

Rabbits

In a 21-day study that complied with GLP, undiluted temephos (purity, 93.1%) was administered to the clipped skin of groups of 12 male and 12 female New Zealand White rabbits (half of which had abraded skin) for 6 h/day (with site of exposure wrapped), 5 days/week for 3 weeks at volumes to achieve doses of 9, 25, 50 and 100/75 mg/kg bw. The group at the highest dose received 100 mg/kg bw for the first 2 weeks and 75 mg/kg bw for the last week. After the treatment period, all of the animals at the highest dose and six animals (three with abraded skin and three with non-abraded) of each sex from each of the other groups were kept for a 14-day observation period before being killed. The other animals were killed at the end of the treatment period. Dermal reactions and clinical condition were recorded daily. Body weights and feed consumption were recorded weekly. Blood samples for haematology and clinical chemistry (including plasma cholinesterase activity) were taken before treatment, on day 21 and at termination. Necropsies were performed on all animals. Selected organs were weighed, brain cholinesterase activity was measured and a wide range of tissues was taken for microscopic examination.

There was a high rate of treatment-related clinical signs and mortality at doses of 50 mg/kg bw or greater. Body-weight gains were also reduced at these doses. There were signs of dermal irritation in all treated groups, with a dose-related severity. Erythrocyte count, haematocrit (erythrocyte volume fraction) and haemoglobin were decreased at 50 mg/kg bw or greater. Albumin and total protein were decreased at the top dose only. Plasma cholinesterase was inhibited at all doses. Brain cholinesterase was inhibited at doses of 50 mg/kg bw or greater. Plasma and brain cholinesterase activities had returned to normal after, respectively, 10 days and 14 days recovery. There were no treatment-related effects on organ weights, gross pathology or histopathology. As plasma cholinesterase activity was inhibited at all doses, no NOEL was identified in this study. However, the NOAEL was 25 mg/kg bw on the basis of effects on haematology and brain cholinesterase activity that were seen at 50 mg/kg bw or greater (Thompson & Rao, 1981).

2.3 Long-term studies of toxicity and carcinogenicity

Rats

A non-GLP combined study of the long-term oral toxicity and carcinogenicity of temephos (purity, 93.5%) was performed with Sprague-Dawley rats. The study incorporated an in-utero phase of exposure. Dosing with diets containing temephos at 100 ppm began on day 12 of pregnancy. At weaning, the offspring were randomly divided into three groups of 60 males and 60 females that were given diets containing temephos at 10, 100 or 300 ppm for 24 months. These dietary concentrations were equivalent to doses of approximately 0.5, 5 and 15 mg/kg bw per day. An additional group of 60 male and 60 female rats from the same supplier received no temephos in utero or in life and were used as controls. Feed intake and body weights were measured weekly during the first 3 months, and during weeks 25, 53, 79, and 104. Urine analysis and haematology were conducted on five males and five females from each treatment group at 6 weeks, 3 months, 6 months and at the conclusion of the study. Blood clinical chemistry was performed on five rats of each sex from each group at the end of the study only. All rats were autopsied, selected organs were weighed and a wide range of organs were examined microscopically. There were no treatment-related effects on survival, body-weight gain, haematology, urine analysis, clinical chemistry, gross pathology, organ weights or histopathology. The treatment did not cause an increase in the incidence of any tumour type. The NOAEL was 300 ppm, equivalent to 15 mg/kg bw per day, the highest dose used (Tegeris, 1977; Shaffer, 1978).

2.4 Genotoxicity

Temephos has been tested for genotoxicity in vitro in several assays, which are summarized in Table 6. Certificates of compliance with GLP were supplied with most of the reports (Allen, 1986, Traul, 1988, Thilagar, 1986 and Barfknecht, 1986), but Brusick (1976) was performed before the requirement to comply with GLP. All except one of the tests was performed using the same batch of temephos (lot No. AC 5105-43; purity, 94.7%). All the assays (apart from the study using a primary culture of hepatocytes, which did not require an external metabolizing system) were performed in the presence and absence of metabolic activation from S9 mix from the livers of rats that had been treated with Araclor 1254. All of the tests gave negative results for genotoxicity in vitro. No studies of genotoxicity in vivo were available.

Table 6. Results of studies of genotoxicity with temephos in vitro

End-point	Test object	Concentration	Purity (%)	Result	Reference
Reverse mutation	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 and *Escherichia coli* WP2 *uvrA*-	Up to 5000 µg/plate	94.5	Negative[a]	Allen (1986)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100 and *Saccharomyces cerevisiae* D4	Up to 100 µg/plate	90	Negative[a]	Brusick (1976)
HGPRT gene mutation	CHO K[1] BH[4] cells	Up to 5000 µg/ml	94.5	Negative[a]	Traul (1988)

Chromosomal aberration	CHO K-I cells (ATCC No CCL 61) with harvest times at 10 h and 20 h	Up to 7.5 µg/ml	94.5	Negative[a]	Thilagar (1986)
DNA repair (unscheduled DNA synthesis)	Rat primary hepatocytes	Up to 5000 µg/ml	94.5	Negative	Barfknecht (1986)

[a] In the presence and absence of S9 mix (9000 × *g* supernatant fraction from liver of rats treated with Araclor 1254).

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

A non-standard non-GLP one-generation study of the effects of temephos on reproduction was performed in Sherman-strain rats. A brief report of the study stated that groups of rats were given diets containing temephos (purity, 90%) at a concentration of 0 or 500 ppm, equivalent to pure temephos at a dose of 22.5 mg/kg bw per day. Fifteen litters were produced from 15 matings of the group given temephos. There were no treatment-related effects on litter size, viability of young or incidence of congenital defects. After 48 days of dosing with temephos, the mothers' mean erythrocyte cholinesterase activity was inhibited by 90% when compared with that of the untreated controls. Erythrocyte cholinesterase was inhibited by 30% in the pup aged 21 days in the treated group (Gaines et al., 1967).

A three-generation study of reproductive toxicity with temephos was performed in CFE Albino rats. The protocol did not comply with GLP, nor did it conform to modern standards. For the F_0 generation, groups of 24 females and 12 males were fed diets containing temephos (purity 87.1%) at a concentration of 0, 25 or 125 ppm for 8 weeks before mating and during 10 days of mating. The doses were equivalent to 0, 1.1 and 5.4 mg/kg bw per day of pure temephos. The pups of each subsequent generation were weaned directly onto the same diets as their parents. For the F_1 and F_2 generations, 16 pairs (one male and one female) were chosen for each dose and mated when aged 14–17 weeks (F_1 generation), 16–20 weeks (first mating of F_2 generation) or 31–36 weeks (second mating of F_2 generation). Body weights were measured only until the first mating of each generation and food consumption was measured only for parents in the F_0 and F_1 generations and these data were not examined statistically. Any effects on the mating and/or reproductive performance of the parent animals were reported. Pups were sexed and evaluated for health and viability. Gross pathological examinations were performed on all parents and offspring. More detailed examination was performed only on the first litter of offspring of the F_2 parents (i.e. pups of the F_{3i} generation), with the two weakest pups from each litter being set aside at age 21 days, one being examined microscopically for visceral defects and histopathology and the other being examined for skeletal defects.

Treatment with temephos had no adverse effect on reproduction or lactation in any of the generations studied. Litter size, fertility index, gestation index, lactation index and the gross appearance of the pups were not affected by treatment. Mean pup weights were generally higher than values for concurrent controls for the group at 25 ppm and lower than controls for the group at 125 ppm, but the effects were slight and were not considered to be treatment-related. No treatment-related effects on visceral or skeletal abnormalities were observed. Microscopic examination of pups of the F_{3i} generation showed a high incidence of pups having a large amount of splenic extramedullary haemopoiesis in the control group and in the group at 125 ppm, but this was not regarded as a

treatment-related effect. The NOAEL for reproductive effects was the highest dose used, 125 ppm, equivalent to 5.4 mg/kg bw per day (McNerney et al., 1968).

(b) Developmental toxicity

Rabbits

In a study of developmental toxicity, which did not comply with GLP, groups of 15–16 pregnant New Zealand White rabbits were given technical-grade temephos (purity, 90.4%) at a dose of 3, 10, or 30 mg/kg bw per day by oral gavage during days 6–18 of gestation. A control group of 21 pregnant rabbits was not given temephos. On day 29 of gestation, the pregnant rabbits were killed and autopsied. Brain, liver and kidneys were weighed and preserved for possible future examination. The uterine contents were examined for implantation sites, live and dead fetuses and resorption sites. The uterus and ovaries were preserved for possible future examination. The fetuses were examined for external abnormalities, weighed and measured (crown to rump length). Autopsies were performed to examine the thoracic and abdominal viscera of the fetuses. The fetuses were then skinned and prepared for skeletal examination.

During the course of the study, five adults in the control group and one in the group at 3 mg/kg bw per day died of causes unrelated to the oral toxicity of temephos (mostly intubation accidents). There were no treatment-related effects on the body-weight gain or absolute organ weights of the adults, but kidney weight relative to body weight in the group at 30 mg/kg bw per day was significantly lower than the control value. The tissues were not examined microscopically, so it was not possible to tell if the effect on relative kidney weight was associated with any histopathology. Examination of the uterine contents revealed no treatment-related adverse effects. The treatment had no effect on the type or number of abnormalities of the soft tissues or skeleton. It was concluded that doses of up to 30 mg/kg bw per day did not produce embryotoxicity, fetotoxicity or teratogenicity in rabbits. The NOAEL was 10 mg/kg bw per day on the basis of reduced relative kidney weight seen in pregnant adults at 30 mg/kg bw per day. The NOEL for developmental toxicity was the highest dose administered, 30 mg/kg bw per day (Beliles & Makris, 1978).

2.6 Special studies: neurotoxicity in hens

The potential of temephos to cause delayed neurotoxicity in mature White Leghorn chickens was investigated in a study that complied with GLP. Ninety chickens (sex not stated) were given intramuscular injections of atropine sulfate at a dose of 10 mg/kg bw. The birds were then divided into three groups: test group, 60 birds; treated control group, 15 birds; and positive control group, 15 birds. One hour after the initial injection of atropine and again 21 days later, the test birds were each given temephos (purity not reported) at an oral dose of 550 mg/kg bw and the birds in the positive-control group were given tri-ortho-tolyl phosphate (TOTP) at an oral dose of 500 mg/kg bw. Further antidote doses of atropine at 30 mg/kg bw were given 1.5, 2.5, 4, 5 and 6.5 h after the first dose and a similar dosing regime of atropine was given before and after the second dose. The birds were observed daily for mortality and neurotoxicological signs for 21 days after each dose. All birds that died during the study were necropsied and at the end of the study the survivors were killed for necropsy, with particular attention being paid to examination of the nervous system. Numerous sections of the brain, the spinal cord and the sciatic nerves were examined microscopically, using standard haematoxylin-eosin staining and Luxol Fast Blue staining for myelin.

Twenty-one of the 60 birds in the test group died during the first 21 days of the study. After the second dose of temephos, 5 out of 32 birds died. Signs of toxicity included severe lethargy, hypersalivation, ataxia and anorexia. Most of the survivors recovered by day 8 after dosing and all appeared well by 21 days after each dose. There were no clinical signs of delayed neurotoxicity in any

of the birds treated with temephos. Microscopic examination of central and peripheral nerve tissues revealed no treatment-related lesions. It was concluded that temephos at a oral doses of 550 mg/kg bw caused no neurotoxicity or delayed toxicity (Fletcher & Leonard, 1986).

In a study to investigate the potential of temephos to cause neurotoxicity, which did not comply with GLP, groups of six adult hens (breed not stated) were given temephos (purity, 99.9%) as a single oral doses at 0 (untreated controls), 227, 455, 823, 1137, 1422 or 1705 mg/kg bw by gavage. A positive-control group was given TOCP at a dose of 450 mg/kg bw. The animals were observed over 21 days. On day 21, the surviving birds in the groups at 227 and 455 mg/kg bw were given a second dose of temephos at 227 or 455 mg/kg bw, after being protected with pyridine-2-aldoxime methane sulfonate (PAM) at a dose of 43 mg/kg bw and atropine at 6.5 mg/kg bw. An additional group, also pre-treated with PAM and atropine, were given a single dose of temephos at 433 mg/kg bw. This group and the groups receiving a second dose were observed for a further 21 days. The hens were observed for signs of neurotoxicity six times per day and for other clinical signs on a daily basis. All birds were killed at the end of the observation periods, but histopathological examinations were performed on only three hens from the positive-control group and three hens from the group given two doses of temephos at 455 mg/kg bw.

Mortality in the group at 227 mg/kg bw was no greater than in the untreated control group. The single-dose oral LD_{50} calculated from the results for mortality in this study was 579 mg/kg bw. There was no treatment-related effect on body-weight gain. Most (five out of six) of the birds in the positive-control group became ataxic, but none of those given temephos became ataxic. While birds given temephos became lethargic and some exhibited tremors, surviving birds recovered within 72 h of dosing and no further signs of toxicity were seen. The microscopic examination of tissues from the birds given temephos at 455 mg/kg bw showed no abnormalities, but birds in the positive-controls group had histopathological lesions in the mid-brain, spinal cord, peripheral nerve, lungs and skeletal muscle.

The Meeting concluded that single oral doses of temephos at up to 1705 mg/kg bw or two oral doses at up to 455 mg/kg bw did not produce neuropathy in hens (Ross et al., 1976).

In a study of the potential of temephos to cause demyelination, which did not comply with GLP, groups of eight adult White Leghorn hens were given diets containing temephos (purity, 87.1%) at a concentration of 230, 460 or 920 ppm (approximately equivalent to 11, 23 and 46 mg/kg bw per day). A negative-control group of six hens received diet containing no temephos and a positive-control group of six hens received TOCP at 4000 ppm. After 30 days, four hens from the group at 920 ppm and two from each control group were killed for autopsy, with samples of brain, thoracic spinal cord and sciatic nerve being taken for microscopic examination (stained with osmic acid to highlight myelin). The remaining birds were then given basal diet for recovery periods of 1 week (groups at 230 and 460 ppm) or 4 weeks (group at 920 ppm), but were not autopsied.

Treatment with temephos caused no deaths, but four of the birds in the positive-control group died. During the treatment period, there was significant body-weight loss in birds fed temephos and in the positive-control group, and there was some recovery of body weight when treatment was ceased for 1–4 weeks. Feed intake was not measured, but it was noted that birds treated with temephos and birds in the positive-control group generally ate less diet during the treatment period than did birds in the negative-control group. The hens given temephos at 920 ppm had diarrhoea for the first 2 weeks of the study, but the temephos-treated birds otherwise appeared in good health throughout the study. In contrast, birds in the positive-control group suffered muscle weakness and/or paralysis from 2 weeks until termination. Microscopic examination of the sampled nervous tissues did not find any myelin loss in birds in the negative-control group or in those given temephos at 920 ppm for 30 days. Microscopic findings were not presented for the birds given temephos at 230 or 460 ppm. The two birds in the positive-control group that were examined showed myelin loss and/or myelin damage in the brain, thoracic spinal cord and sciatic nerve.

The Meeting concluded temephos at dietary concentrations of up to 920 ppm (46 mg/kg bw per day) for 30 days did not cause demyelination of nerves of hens (McNerney & Levinskas, 1967).

3. Observations in humans

3.1 Studies in volunteers

Temephos was given orally to human volunteers in two experiments designed to determine the dosage needed to produce depression of cholinesterase activity in plasma and in erythrocytes. Twenty-eight healthy male volunteers (aged between 22 and 44 years) were selected from a Puerto Rican prison. For the first week of the study, all the men were given daily doses of milk containing no added temephos; during this week, three blood samples were taken for measurement of baseline activities of cholinesterase in plasma and in erythrocytes. At the end of the first week, 20 of the men were randomly assigned to two groups of 10 men for the first experiment. One group received daily glasses of milk containing technical-grade temephos of unreported purity and the other group received milk containing no temephos and served as a control group. The dosage of temephos was initially 2 mg/person per day and was doubled every 3–4 days for 4 weeks, when the dose reached 256 mg/person per day (given for 5 days). Dosing with temephos ceased after 4 weeks and the men were observed for a further 3 weeks, during which they received no temephos. Blood samples were taken three times per week for measurement of plasma and erythrocyte cholinesterase activity. Urine was collected while the men received the highest dosage and during the 3 weeks after the treatment period; measurements of ether-extractable organic phosphorus were made from the urine. The high concentrations of temephos in the milk given to the experimental group in the final week of the treatment period were sufficiently high to flavour the milk, so that the volunteers became aware of whether they were in the control or test group. One man felt nauseous, but apart from this there were no treatment-related adverse reactions. Activities of cholinesterase in the plasma and erythrocytes of the treated group were no different from the values for the control group. The mean concentration of ether-extractable organic phosphorus was 1.15 ppm of temephos equivalents at the end of the treatment period.

In the second experiment of the study, a control group of nine men and an experimental group of nine men were chosen. None of the men in the experimental group had previously received temephos. The control group was given milk containing no temephos and the experimental group was given milk containing temephos at a dose of 64 mg/person per day for 4 weeks. During the treatment period, blood samples were taken twice per week and an additional sample was taken 5 days after the last dose. Urine samples were collected during weeks 1, 3 and 4 of dosing and for 3 weeks after the last dose. Cholinesterase activity was measured in plasma and erythrocytes and ether-extractable organic phosphorus was measured in the urine samples. One man developed a transitory fever, with coryza and myalgia, which disappeared after 3 days, and 4 other men briefly suffered headache and coryza. The affected men came from the control group as well as the treated group, with no clear effect of treatment being evident. At no time during the experiment were there any significant effects on cholinesterase activities in plasma and erythrocytes. Mean urinary ether-extractable organic phosphorus rose to 1.8 ppm of temephos equivalents in the treated group at 21 days and then went down to 1.25 ppm at 28 days.

The Meeting concluded that the volunteers tolerated of temephos at a dose of 256 mg/person per day (4.27 mg/kg bw per day for a 60-kg person) for 3–4 days (preceded by progressively escalating doses before this) or a dose of 64 mg/person per day (1.1 mg/kg bw per day) for 4 weeks (Laws et al., 1967).

3.2 Field studies

A 19-month study of a village community of approximately 2000 people was conducted. Temephos was added to all cisterns and other potable water available to the community. The treatment occurred once per month and consisted of 1% temephos adsorbed to sand in sufficient quantity to achieve a calculated

concentration of 1 ppm. Only one sample ever contained temephos at a concentration of greater than 0.5 ppm. This was attributed to the combined effects of adsorption, solubility and dilution over time. No significant change was measured in either plasma or erythrocyte cholinesterase activities of the villagers at any time during the study. Urinary excretion of temephos reached a steady state after 4 months. No illness attributable to the insecticide occurred and all eight babies born were normal (Laws et al., 1968).

A 2% formulation of temephos in pyrax powder was applied to participants and their bedding from a shaker (57 g, equivalent to 1.1 g of temephos) or to clothed subjects from a powder duster (31 g, equivalent to 0.62 g of temephos). The treatment was reported to be safe and effective (Steinberg et al., 1972).

3.3 Health monitoring of workers

No data were available.

Comments

Biochemical aspects

When given to rats as an oral dose, temephos was rapidly absorbed. At least 40% of the administered dose was absorbed into the blood plasma. Clearance was rapid (mostly within 48 h), with about 40% of an orally administered dose being excreted in the urine and about 60% recovered in the faeces. Very little of the orally administered dose remained in tissues, but most (about 3% of the administered material) was in adipose tissue.

Metabolism in rats is by *S*-oxidation to form the primary toxicant, temephos sulfoxide, and by carboxylesterase-mediated hydrolysis to form 4,4'-thiodiphenol. Temephos and these primary metabolites can undergo secondary metabolism by glucuronidation or sulfation to form conjugates.

Toxicological data

Temephos was of low acute oral toxicity in rats (LD_{50}, 4000–13000 mg/kg bw) and the mouse (LD_{50}, 2062 mg/kg bw).

Temephos did not cause irritancy to rabbits' eyes or to the skin of rabbits or guinea-pigs. It was not a skin sensitizer when tested on guinea-pigs in the Buehler test.

In short-term studies with temephos administered in the diet or by gavage in rats (28–92 days), rabbits (30–35 days) and dogs (90–129 days), acetylcholinesterase activity in erythrocytes and, in some instances, in the brain was measured and animals were observed for clinical signs. The overall NOAEL for clinical signs was 10 mg/kg bw per day as derived from a study in rats treated by gavage for 28- and 44-days and from a study in rabbits treated by gavage for 35 days. This NOAEL is supported by the absence of clinical signs at 5.4 and 30 mg/kg bw per day, the highest doses tested, in the multigeneration study in rats and in a study of developmental toxicity in rabbits treated by gavage, respectively. Additional support is provided by the presence of mild signs in dogs given diets containing temephos at a concentration of 500 ppm for about 11 weeks, approximately equivalent to 25 mg/kg bw per day. The NOAEL for biologically significant (i.e. 20% greater than control values) inhibition of brain acetylcholinesterase activity was 54 ppm (2.3 mg/kg bw per day) in a 90-day dietary study in rats. In a 90-day dietary study in dogs, "marked" inhibition (no control values provided) in brain and > 95% inhibition of erythrocyte acetylcholinesterase activity were reported after treatment at 500 ppm (25 mg/kg bw per day), and there was no inhibition of erythrocyte acetylcholinesterase activity at 18 ppm (about 1 mg/kg bw per day). The overall NOAEL for biologically significant (i.e. 20% greater than control values) inhibition of erythrocyte acetylcholinesterase activity was 1.8 mg/kg

bw per day in a 99-day dietary study in rats. Occasional and inconsistent reductions of erythrocyte acetylcholinesterase activity observed at lower doses in some studies in rats were not considered to be significant. The Meeting noted that between 80% and more than 90% inhibition of erythrocyte acetylcholinesterase activity was not associated with clinical signs of cholinergic toxicity in a 99-day dietary study in rats or in a limited 129-day dietary study in dogs, and this suggested that inhibition of erythrocyte acetylcholinesterase activity was not an appropriate indicator of inhibition of the activity of acetylcholinesterase in the peripheral nervous system. Consequently, the Meeting considered that the critical end-point for human risk assessment was inhibition of brain acetylcholinesterase activity and the NOAEL was 2.3 mg/kg bw per day.

In a study in human male volunteers who were prisoners, 10 men were given temephos at a dose of 1.1 mg/kg bw per day for 4 weeks and 9 men took temephos at a dose of 4.27 mg/kg bw per day for 5 days. There was no inhibition of cholinesterase activity in the plasma or in erythrocytes. This study in human volunteers who were prisoners was considered to be ethically acceptable according to the standards of the time it was performed (1967), although it would not be acceptable by current standards applied to new studies. The Meeting considered that the doses and the outcomes in this study in humans were not sufficiently well described for the results of this study to be used in isolation to set an ADI or an acute reference dose (ARfD).

Hepatotoxicity was inconsistently seen in a series of briefly reported experiments in rabbits. However, there was no evidence of any hepatotoxicity at doses of up to 30 mg/kg bw per day in a well-conducted and well-reported study of developmental toxicity in rabbits.

In a long-term combined study of toxicity and oncogenicity in rats, no adverse effects on neoplastic or non-neoplastic pathology were found at any dietary dose tested, up to the highest dose of 15 mg/kg bw per day. Cholinesterase activities were not measured in this study.

Temephos gave uniformly negative results in an adequate range of tests for genotoxicity in vitro and in vivo. The Meeting concluded that temephos is unlikely to be genotoxic.

Studies of reproductive toxicity in rats showed that temephos did not adversely affect reproduction when given as oral doses of up to 125 ppm (5.4 mg/kg bw per day) for up to three generations. In a one-generation study, temephos at a dose of 500 ppm (22.5 mg/kg bw per day) inhibited erythrocyte cholinesterase in mothers (90%) and in 21-day-old pups (30%), but other doses were not tested. There was no developmental toxicity or hepatotoxicity in rabbits given temephos at oral doses of up to 30 mg/kg bw per day.

Studies in hens showed that temephos did not have the potential to cause organophosphate-induced delayed neuropathy and did not cause demyelination of nerves.

Although, for the purposes of vector control, temephos is used at a concentration of up to 1 mg/l in drinking-water, only one report of an investigation of possible effects in exposed people was available. Approximately 2000 people were exposed to drinking-water containing temephos for 19 months without any adverse effects on plasma or erythrocyte cholinesterase activity. No illness attributable to the treatment was seen and all eight babies born during the study period were normal. The drinking-water was treated monthly with temephos. The intended concentration of 1 ppm was not achieved and only one sample contained temephos at a concentration of more than 0.5 ppm.

Toxicological evaluation

Temephos is recommended by WHO for addition to potable water as larvicide treatment at an application rate not exceeding 1 mg/l. Assuming that an adult weighing 60 kg would consume 2 l per day of drinking-water containing temephos at 1 mg/l, this would be equal to an oral exposure of 0.033 mg/kg bw. However, given the limited solubility of temephos in water, incomplete dissolution in drinking-water would be expected and this could result in actual exposures

being appreciably less than this estimate. Consequently, 0.033 mg/kg bw per day was regarded as a worst-case upper limit of exposure.

Some of the studies that were critical to the assessment were of poor quality. The Meeting considered that the database was insufficiently robust to serve as the basis for establishing an ADI or an ARfD for temephos.

The Meeting concluded that the relevant NOAEL for human risk assessment is 2.3 mg/kg bw per day on the basis of inhibition of brain acetylcholinesterase activity in rats. This NOAEL provides a margin of exposure (MoE) from the estimated oral exposure derived from drinking-water treated with temephos of about 70. The MoE for clinical signs and the, possibly secondary, effects on development and reproduction are in the range of > 160 (highest dose tested in rat multigeneration study) to 900 (study of developmental toxicity in rabbits). In addition, reassurance is provided by the MoEs of 130 and 33 provided by the absence of clinical signs and erythrocyte cholinesterase inhibition in the poorly described study in volunteers treated for 5 or 14 days, respectively.

In addition to this safety assessment, the concerned WHO programmes will consider efficacy of the treatment and additional relevant exposure scenarios before further recommending such a treatment and deriving water-guideline values.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	28-day and 44-day study of toxicity[b]	Clinical signs	10 mg/kg bw per day	100 mg/kg bw per day
	90-day study in rats[a]	Inhibition of acetylcholinesterase in brain	2.3 mg/kg bw per day[d]	—
	2-year study of toxicity and carcinogenicity[a]	Carcinogenicity (cholinesterase activity not measured)	300 ppm (15 mg/kg bw per day)[d]	—
	Three-generation study of reproduction[a]	Maternal	125 ppm (5.4 mg/kg bw per day)[d]	—
		Offspring	125 ppm (5.4 mg/kg bw per day)[d]	
Rabbit	35-day study of toxicity[b]	Clinical signs	10 mg/kg bw per day[d]	—
	Study of developmental toxicity[b]	Maternal	30 mg/kg bw per day[d]	—
		Developmental	30 mg/kg bw per day[d]	—
Dog	90-day study of toxicity[a]	Inhibition of acetylcholinesterase in erythrocytes	18 ppm (0.9 mg/kg bw per day)	700/500 ppm (35/25 mg/kg bw per day)
		Inhibition of acetylcholinesterase in brain	—	700/500 ppm (35/25 mg/kg bw per day)[e]
	129-day study of toxicity[a]	Inhibition of acetylcholinesterase in erythrocytes	10 ppm (0.6–0.8 mg/kg bw per day)	50 ppm (3–4 mg/kg bw per day)
Chickens	Neurotoxicity[b]	No organophosphate-induced delayed neurotoxicity observed	1705 mg/kg bw[e]	—

Humans	Investigation of clinical signs and effects on cholinesterase activities in erthrocytes and plasma[c]	No adverse effects reported	1.1 mg/kg bw per day for 4 weeks or 4.27 mg/kg bw per day for 5 days[e]	—

[a] Dietary administration
[b] Oral gavage
[c] Administration in a drink
[d] Highest dose tested
[e] Only dose at which measurements of the critical end-point were made.

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	At least 40% of administered dose absorbed.
Distribution	Most of the retained residues were in the fat (about 0.5% of administered dose)
Potential for accumulation	Low
Rate and extent of excretion	Most of the administered oral dose recovered in the urine and faeces within 48 h. About 40% in urine.
Metabolism in mammals	By hydrolysis and *S*-oxidation plus conjugation.
Toxicologically significant compounds	Temephos and temephos sulfoxide
Acute toxicity	
Rat, LD_{50}, oral	4000 to 13 000 mg/kg bw
Rat, LD_{50}, dermal	2000 to > 4000 mg/kg bw
Rat, LC_{50}, inhalation	> 4.79 mg/l
Rabbit, skin irritancy	Non-irritant
Rabbit, eye irritation	Non-irritant
Guinea-pig, skin sensitization (test method)	No skin sensitizing potential (modified Buehler test)
Short-term studies of toxicity	
Critical effects	Inhibition of brain acetylcholinesterase activity
Lowest relevant oral NOAEL	2.3 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	25 mg/kg bw per day (rabbit)
Genotoxicity	
	Not genotoxic (on the basis of tests in vitro)
Long-term studies of toxicity and carcinogenicity	
Critical effects	No adverse effects detected at any dose tested (cholinesterase activities not measured)
Lowest relevant oral NOAEL	15 mg/kg bw per day (rats, highest dose tested)
Carcinogenicity	Not carcinogenic in rats
Reproductive toxicity	
Reproductive target/critical effects	None
Lowest relevant reproductive NOAEL	5.4 mg/kg bw per day (rats, highest dose tested)
Developmental critical effect	None

Lowest relevant developmental NOAEL	10 mg/kg bw per day for maternal toxicity 30 mg/kg bw per day (highest dose tested) for developmental effects
Neurotoxicity studies in hens	
Lowest relevant neurotoxicity NOAELs	1705 mg/kg bw (highest dose tested caused no neuropathy)
Observations in humans	
Volunteer studies	No effects on plasma or erythrocyte cholinesterase activity at 1.1 mg/kg bw per day for 4 weeks, or at 4.27 mg/kg bw per day for 5 days

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THIABENDAZOLE (addendum)

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Explanation

Thiabendazole, the International Organization of Standardization (ISO) approved name for 2-(4-thiazolyl)-1*H*-benzimidazole (CAS No. 148-79-8), is a benzimidazole compound used as a systemic fungicide in agriculture. Thiabendazole is also used as a broad-spectrum anthelmintic in various animal species, for control of parasitic infestations in humans, and in materials protection (as a preservative in adhesives, coatings, paper, textiles and paints).

The toxicology of thiabendazole was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1970 and 1977. In 1977, the Meeting established an acceptable daily intake (ADI) of 0–0.3 mg/kg bw on the basis of the absence of effects at 3 mg/kg bw per day in a 6-month study in human volunteers, and a safety factor of 10.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the toxicology of thiabendazole in 1992, 1997 and 2002. In 1997, the Committee established an ADI of 0–0.1 mg/kg bw. In 2002, the Committee established a conservative acute reference dose (ARfD) of 0.1 mg/kg bw based on no-observed-adverse-effect levels (NOAELs) for haemolytic anaemia in repeat-dose studies of toxicity in rats and dogs, in view of the lack of more appropriate acute toxicity data.

After the JECFA evaluation of thiabendazole in 2002, the sponsor conducted several studies of acute toxicity with thiabendazole administered by gavage or in the diet that were designed specifically to derive an ARfD and to determine the pharmacokinetic events that apply under these exposure scenarios. At the request of the Codex Committee on Pesticide residues (CCPR) at its Thirty-eighth Session (Codex Alimentarius Commission, 2006), thiabendazole was re-evaluated at the present Meeting in order to establish an ARfD. The Meeting also reviewed some relevant data from previous evaluations (studies of reproductive toxicity and developmental toxicity, as well as data from humans).

All new studies with thiabendazole were certified as complying with good laboratory practice (GLP).

Evaluation for acute reference dose

1. Biochemical aspects: comparative pharmacokinetics

To better understand the neuroactive effects observed in the studies of toxicity with single doses, several experiments were conducted to relate the observed biological activity of thiabendazole to the pharmacokinetic parameters observed after dosing by gavage or in the diet. This study was evaluated for the first time by the present Meeting.

This study investigated the comparative bioavailability of thiabendazole after a single dose of [^{14}C]thiabendazole (radiochemical purity, > 95%; specific activity, 2.02 MBq/mg) or the equivalent dietary dose over 24 h to CD®:Sprague-Dawley rats. In the gavage experiments, a single dose at 20 or 50 mg/kg bw was administered as a suspension in 0.5% w/v aqueous carboxymethylcellulose to fasted (50 mg/kg bw only) and non-fasted rats. In a separate group whose treatment was designed to mimic the first mouthful of food for the purposes of ARfD determination, a single dose of an aqueous slurry of diet containing [^{14}C]thiabendazole at a concentration of 500 ppm (equal to a mean achieved dose of 0.62 mg/kg bw in males and 0.61 mg/kg bw in females)was given by gavage to non-fasted rats; these rats were then given access to diet containing unlabelled thiabendazole (purity, 99.5%) at a concentration of 500 ppm for 24 h, followed by control diet. Rats in the dietary-kinetics treatment group were given access to diet containing [^{14}C]thiabendazole at a nominal concentration of 200 or 500 ppm, equal to mean achieved doses of 15 mg/kg bw (males and females) and 49 mg/kg bw (males) or 47 mg/kg bw (females) at the lower and higher doses, respectively) for 24 h. In all groups, bioavailability was determined by analysis of one blood sample taken at different times after dosing. There are no specific guidelines for studies of this type, but the study was conducted in accordance with the principles of OECD TG 417 (1984) and was considered to be acceptable.

No adverse clinical signs were recorded in any of the treatment groups. The peak concentration of radioactivity (C_{max}), time of peak concentration (T_{max}), area under the curve (AUC) and half-life in whole blood and in plasma are reported in Table 1 and 2, respectively.

The comparative blood profiles for rats given a single oral dose at 50 mg/kg bw by gavage showed that the presence of diet influenced the rate and extent of absorption of thiabendazole from an aqueous suspension dose. Irrespective of the dietary status of rats, absorption of thiabendazole was rapid. However, in non-fasted rats absorption was more protracted and plasma C_{max} values were lower than in fasted animals. The blood [^{14}C] profiles did not show any marked difference between the sexes. The apparently slow terminal rate of elimination of radioactivity from blood was shown to be attributable to the binding of radioactivity to erythrocytes. The AUC values were greater in the fasted than in the non-fasted animals, which is consistent with the peak concentrations seen in these groups. The values for males were also noticeably greater than for females.

Feeding rats with diets containing thiabendazole at a concentration of 500 ppm confirmed the continued absorption of thiabendazole from diet, with blood concentrations of radioactivity rising over at least 12 h after the presentation of diet, followed by fairly constant blood concentrations until the removal of diet, after which blood radioactivity concentrations began to decline. The slow terminal elimination was again associated with the binding of radioactivity to erythrocytes.

The C_{max} for blood concentrations of radioactivity after exposure to radiolabelled thiabendazole at a dose of 50 mg/kg bw by gavage was about twice the value after dietary exposure for 24 h at 500 ppm (total dose of 49 mg/kg bw for males and 47 mg/kg bw for females). However, the half-lives after exposure by either regimen did not greatly differ.

Table 1. Blood concentrations of radioactivity in rats given a single dose of [^{14}C]thiabendazole by gavage or in the diet

Dose/dietary concentration	Treatment	Sex	Pharmacokinetic parameter			
			C_{max} (µg equivalents/g)	T_{max} (h)	AUC (µg equivalents per h/g)	Half-life (h)
50 mg/kg bw	Non-fasted	Male	13.49	5.05	638.2	68.01
		Female	11.69	4.36	464.5	74.24
	Fasted	Male	21.93	4.15	898.9	132.07
		Female	23.85	2.11	705.9	84.38
500 ppm	Dietary slurry	Male	0.123	0.58	1.363	65.94
		Female	0.102	0.67	1.195	56.75
	Dietary	Male	6.911	13.17	364.0	91.64
		Female	5.841	12.22	267.1	72.33
20 mg/kg bw	Non-fasted	Male	9.47	0.510	178.9	42.37
		Female	11.45	1.010	167.6	34.42
200 ppm	Dietary	Male	1.70	11.98	48.70	45.91
		Female	1.42	12.05	42.28	29.13

From Duerden & Jones (2005)
AUC, area under the curve; C_{max}, peak concentration, T_{max}, time of peak concentration.

Results at the lower doses of 20 mg/kg bw by gavage and 200 ppm in the diet (administered over 24 h, delivering a total dose of 15 mg/kg bw) were compared to determine the linearity of the respective pharmacokinetic parameters. Dosing by gavage again resulted in very rapid absorption, with the C_{max} for radioactivity being attained at approximately 1 h with the characteristic rapid distribution half-life, followed by a much slower terminal half-life elimination. In contrast, after an equivalent dietary dose of 200 ppm, radioactivity was steadily absorbed throughout the exposure interval, with a much lower C_{max} value (about tenfold lower). Again, there was no marked sex difference in either blood or plasma profiles. The comparative blood and plasma curves confirmed the binding of radioactivity to blood cells and also clearly showed that plasma concentrations declined to very low values before the termination of these experiments (at 24 h and 30 h for the groups treated by gavage and with dietary doses, respectively).

Table 2. Plasma concentrations of radioactivity in rats given a single dose of [^{14}C]thiabendazole by gavage or in the diet

Dose	Treatment	Sex	Pharmacokinetic parameter			
			C_{max} (µg equivalents/g)	T_{max} (h)	AUC (µg equivalents per h/g)	Half-life (h)
20 mg/kg bw	Non-fasted	Male	10.17	0.510	46.54	7.37
		Female	12.52	1.010	55.91	8.35
200 ppm	Dietary	Male	1.14	11.98	21.51	9.49
		Female	1.28	12.05	23.21	10.07

From Duerden & Jones (2005)
AUC, area under the curve; C_{max}, peak concentration, T_{max}, time of peak concentration.

In a third set of pharmacokinetic experiments, rats received [^{14}C]thiabendazole at a dose of 500 ppm as an aqueous slurry with diet. The remainder of the daily dietary intake was satisfied by the consumption of diet treated with unlabelled thiabendazole at the same concentration. In essence, this experimental design allowed the fate of the first mouthful of treated diet to be monitored. The

use of this dose form was considered to parallel the creation of such a slurry in the stomach when a rat consumes treated diet and water. The design of this experiment provided an opportunity to follow the absorption of a radiolabelled dietary dose. The requirement for a 10% suspension of diet in water restricted the amount of diet that could be administered. Based upon values for dietary consumption , these rats received an approximate dose of radiolabelled plus unlabelled thiabendazole of 50 mg/kg bw over 24 h. Therefore, this dose was similar to that in rats dosed either by gavage or in dry diet. However, the amount of [^{14}C]thiabendazole administered in the slurry dose was more than 100-fold less than the dose of thiabendazole at 50 mg/kg administered by gavage in 0.5% carboxymethylcellulose. The blood [^{14}C] profiles showed very rapid absorption of radioactivity from thiabendazole with a C_{max} of less than 1 h, followed by a very rapid decline in radioactivity within 2 h after administration, and then by a slower terminal rate of elimination, consistent with observations in the previous groups. Similarly, there was no marked difference between the sexes. These findings confirmed that thiabendazole was rapidly absorbed from the diet. The AUCs for the animals receiving a dose of 500 ppm as dietary slurry and the animals receiving a dietary concentration of 500 ppm were shown to be directly comparable when adjusted for dose. Hence, the fate of the "first mouthful" of radiolabelled diet was indicative of the fate of subsequent dietary intake. The rate of absorption from diet is therefore fast, although not as fast as from a dose in the absence of diet.

In conclusion, an aqueous suspension of thiabendazole was absorbed rapidly when given to fasted and non-fasted rats by oral gavage. However, the extent of absorption was smaller and continued throughout the dietary exposure interval when thiabendazole was administered with diet. At each dose, the comparative gavage and dietary doses were similar (the achieved dose administered in the group receiving a dietary dose at 200 ppm was slightly lower than expected and was attributed to a slightly lower consumption of diet by this group), thereby enabling a direct comparison of blood and plasma radioactivity profiles. C_{max} concentrations of total radioactivity were consistently higher after a gavage dose of an aqueous suspension of thiabendazole than an equivalent dietary dose, indicating that certain effects could be greater if toxicity was determined solely by magnitude of C_{max}. Similarly, AUC values were higher after a gavage dose of a suspension of thiabendazole in carboxymethylcellulose than from a dietary dose. In neither case was there any pronounced difference between the sexes, although AUCs were all higher (7–37% higher depending on the experimental group) in males than in females. After a dose of 50 or 20 mg/kg bw given by gavage to non-fasted rats, the AUC values were consistent with the dose differential. However, in the dietary groups the comparative AUC values were much lower in the group at 200 ppm than in the group at 500 ppm, which was also consistent with the peak concentrations. The half-lives for radioactivity after exposure by either regimen did not differ greatly, indicating that dosing by gavage or in the diet are equally appropriate mechanisms to maintain steady systemic levels of thiabendazole for a sufficient length of time to study its acute hazard potential.

Dietary or gavage studies of acute toxicity (with supporting kinetics) can be equally acceptable for the estimation of hazard potential. However, kinetic studies with dietary slurry provide the best estimate or scenario regarding physiological disposition of compounds after the first mouthful of food containing a high concentration of residue. The administration by gavage of single, high doses of a compound can never mimic human dietary consumption patterns. Nevertheless, this kinetic investigation was conducted to determine the parameters following a plausible, albeit unlikely, high residue exposure scenario (Duerden & Jones, 2005).

2. Toxicological studies

2.1 Studies of toxicity with single doses

These studies were evaluated for the first time by the present Meeting.

In a dose range-finding study, groups of three female CD®:Sprague-Dawley rats were given thiabendazole (purity, 99.4%) at a dose of 0, 100 or 1000 mg/kg bw by gavage on a single occasion, using 0.5% w/v aqueous carboxymethylcellulose as the vehicle. The animals in each group were maintained for 14 days to assess the development of any findings. Clinical observations were recorded at 0.5, 1, 2, 3, 4, 5, 6 and 24 h after dosing and daily thereafter. Body weights and food consumption were measured throughout the study. On day 15, the animals were killed and examined post mortem.

Almost immediately (1–4 h) after dosing at 1000 mg/kg bw by gavage, some clinical signs (sedation, slight piloerection and slight upward curvature of the spine) were observed. Recovery from these initial clinical signs began from 5 h after dosing, with full recovery by 24 h. Although full recovery from the initial clinical effects was apparent, signs indicative of mild systemic toxicity were later seen between days 3 and 8 of the study. Thus, slightly pinched-in sides and upward curvature of the spine were seen again from days 3 to 5, with slight upward curvature of the spine persisting until day 8. Full recovery from these clinical signs was noted by day 9. There were no adverse clinical signs for animals at 100 mg/kg bw. Body-weight loss and reduced body-weight gain were observed between days 1 and 8 for animals (two out of three) at 1000 mg/kg bw. A full recovery in body weight was then seen with these animals overall gaining slightly more weight than the controls. Overall, body-weight gain from days 1 to 15 at 1000 mg/kg bw was similar to controls. There were no effects of treatment on the body weights of animals at 100 mg/kg bw. There was an associated reduction in food consumption among animals at 1000 mg/kg bw on days 1 to 4, with consumption similar to, or slightly higher than, that of controls for the remainder of the study. A slight reduction in food consumption was also present, on day 1 only, for animals at 100 mg/kg bw. However, in the absence of any associated effects on body weight, this was considered not to be of toxicological significance. No abnormalities were observed upon examination post mortem.

A single dose of 1000 mg/kg bw was associated with clinical signs and reduced body weight indicative of mild systemic toxicity, and sedation indicative of a possible neuroactive effect. The time to peak effect for the clinical observations was between 3 and 4 h after dosing. Full recovery from these effects was observed by day 9 and day 15, respectively. The NOAEL was 100 mg/kg bw. Accordingly, the doses chosen for the first study ranged between 100 and 1000 mg/kg bw (Noakes, 2004a).

In the first study, groups of 10 male and 10 female non-fasted CD®:Sprague-Dawley rats were given thiabendazole (purity, 99.4%) at a dose of 0, 100, 200 or 1000 mg/kg bw by gavage on a single occasion using 0.5% w/v aqueous carboxymethylcellulose as the vehicle. Half of the animals in each group were killed after 24 h and the remainder were maintained on study for up to 14 days to assess the development of any findings. Clinical observations were recorded at 0.5, 1, 2, 4 and 24 h after dosing and daily thereafter. Body weights and food consumption were measured throughout the study. In addition, detailed clinical observations, including a qualitative assessment of sensory perception and quantitative assessments of landing-foot splay and muscle weakness, and assessment of motor activity were performed 3 h after dosing, 24 h after dosing and on day 15. Urine samples for clinical chemistry were collected on days 2 and 14. After either 24 h or 14 days, the animals were killed and examined post mortem. Sample of cardiac blood were taken for clinical pathology, selected organs (adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus and uterus with cervix) were weighed and specified tissues were taken for subsequent histopathological examination. The study was performed according to the proposed test guideline document published by JMPR 2000, *Proposed test guideline – single-dose toxicity study by the oral route (for use in establishing acute reference doses for chemical residues in food and drinking water)*[1] .

[1] The document entitled *Guidance document for setting an acute reference dose (ARfD) (prepared by Germany)* from the European Commission, Directorate General for Agriculture VI B II.1, 7199/VI/99 rev. 3, dated 2 August 1999, was also consulted.

At 1000 mg/kg bw, qualitative effects observed in all the test animals included slightly decreased activity in all males at 1 h and in all females at between 0.5–2 h after dosing. Tiptoe gait was observed at 4 h in 1 of 10 males. Full recovery from these effects occurred by 24 h after dosing, except for one male who showed full recovery on day 3. At 100 and 200 mg/kg bw, slightly decreased activity was observed for males and females between 0.5 or 1 and 4 h after dosing on day 1. Tiptoe gait was observed in 2 of 10 males at 200 mg/kg bw between 2 and 4 h after dosing. No adverse clinical signs were present by 24 h after dosing. Compared with controls, body weights were decreased for males and females at 1000 mg/kg bw; the maximum differences occurred on days 4–5 and were 11% and 7% and for males and females, respectively. Full recovery for females and a partial recovery for males were seen by day 15. Correspondingly, food consumption was slightly lower on days 1–5 for males and females, relative to controls. At 200 mg/kg bw, body weights were marginally lower, by about 3%, in males on day 2 only.

During the detailed functional observation battery (FOB), slight tiptoe gait was observed in several treated animals at 3 h after dosing (Table 3).

Slightly decreased activity was also recorded and a slightly reduced righting reflex was seen at 3 h in some animals at 200 or 1000 mg/kg bw. At 24 h after dosing, slightly decreased activity was still present in few males at 1000 mg/kg bw. None of these effects were present on day 15 of the study. Landing-foot splay was reduced for females only at 3 h after dosing and for males and females at 24 h after dosing. By day 15, landing-foot splay values were similar to controls. Motor activity was reduced for males and females treated with 1000 mg/kg bw at 3 h and 24 h after dosing, but was similar to the controls by day 15. At 100 and 200 mg/kg bw, motor activity was reduced for males only, at 3 h after dosing.

Table 3. Results of a detailed functional observation battery (No. of animals affected) in a study of acute toxicity in rats given thiabendazole by gavage

Parameter	Dose (mg/kg bw)							
	Males				Females			
	0	100	200	1000	0	100	200	1000
1–4 h								
Tip to gait	0	3	4	2	0	3	3	3
Decreased activity	0	0	4	8	0	0	0	2
Reduced splay reflex	0	0	0	0	0	0	0	0
Reduced righting reflex	0	0	2	1	0	0	0	0
24 h								
Tip to gait	0	0	0	0	0	0	0	0
Decreased activity	0	0	0	3	0	0	0	0
Reduced splay reflex	0	0	0	0	0	0	0	0
Reduced righting reflex	0	0	0	0	0	0	0	0
Day 15								
Tip to gait	0	0	0	0	0	0	0	0
Decreased activity	0	0	0	0	0	0	0	0
Reduced splay reflex	0	0	0	0	0	0	1	0
Reduced righting reflex	0	0	0	0	0	0	0	0

From Noakes (2004b)

There were no treatment-related changes in haematology parameters measured on days 2 and 14. At 1000 mg/kg bw, and only on day 2, cholesterol and triglyceride concentrations were slightly

higher for males (125% and 172% of that of controls, respectively) and females (125% and 145% of that of controls, respectively), urea concentration was lower for males and females (80% and 67%), alanine aminotransferase activities (73%) and plasma calcium concentration (96%) were slightly lower for males, total plasma protein was slightly higher for males (107%), glucose and phosphorus concentrations were slightly lower for females (73% and 87%, respectively). Urinary pH was slightly higher for males on day 2. No treatment-related blood or urine clinical chemistry changes were observed on days 15 or 14 of the study, respectively. At 200 mg/kg bw, cholesterol concentrations were slightly higher for females (126% of control) and alanine aminotransferase activities (69%) and plasma calcium (96%) were slightly lower for males on day 2 only. There were no effects at 100 mg/kg bw.

Relative liver weights at 1000 mg/kg bw were slightly higher for males and females on day 2 only (111% and 110% of control, respectively). Absolute and relative brain (105% and 106% of control, respectively) and heart weights (118% of control for both) were slightly higher for females on day 15. There were no histopathological findings associated with treatment.

Dose-related effects involving reduced activity, tiptoe gait, landing-foot splay and reduced motor activity were observed in males and females up to 24 h after dosing at 100 or 200 mg/kg bw or up to 3 days after dosing at 1000 mg/kg bw. At the highest dose, there was also a reduction in body weight compared with controls. Recovery from these effects was observed by day 15. The minor reversible blood chemical chemistry changes, in the absence of any treatment-related histopathological findings, were considered not to be of toxicological importance. Similarly, the slight and transient liver-weight effect and the slight brain- and heart-weight effects, observed for females only, in the absence of any histopathological changes in these organs, were considered not to be of toxicological significance. A clear NOAEL was not identified in this study (Noakes, 2004b).

In order to better understand the nature and duration of the reversible neuroactive effects observed in the main single-dose study of toxicity (Noakes, 2004b), and also to establish clear NOAELs for all observation periods, an additional study was conducted. In this second study, groups of 10 male and 10 female non-fasted CD®:Sprague Dawley rats were given thiabendazole (purity, 99.4%) at a dose of 0, 20, 50 or 100 mg/kg bw by gavage on a single occasion using 0.5% w/v aqueous carboxymethylcellulose as the vehicle. Half of the animals in each group were killed after 24 h and the remainder were maintained for up to 14 days to assess the development of any findings. Clinical observations were recorded at 0.5, 1, 2, 4 and 24 h after dosing and daily thereafter. Body weights and food consumption were measured throughout the study. In addition, detailed clinical observations, including a qualitative assessment of sensory perception and quantitative assessments of landing-foot splay and muscle weakness, and assessment of motor activity were performed 3 h after dosing, 24 h after dosing and on day 15. Since haematological parameters were not affected in the main study and clinical chemistry parameters were not considered to be affected in any toxicologically significant way (Noakes, 2004b), appropriate samples, including cardiac blood, were collected and stored, but were not analysed in this study. After either 24 h or 14 days, the animals were killed and examined post mortem. Selected organs were weighed and specified tissues taken and stored; however, histopathological examinations were not conducted. The study was performed according to the proposed test guideline document published by JMPR 2000: *Proposed test guideline – single-dose toxicity study by the oral route (for use in establishing acute reference doses for chemical residues in food and drinking water)*[2].

[2] The document entitled *Guidance document for setting an acute reference dose (ARfD) (prepared by Germany)* from the European Commission, Directorate General for Agriculture VI B II.1, 7199/VI/99 rev. 3, dated 2 August 1999, was also consulted.

At 100 mg/kg bw, slightly decreased/subdued activity was seen during qualitative clinical observations in up to two males and two females on days 1–2, and tiptoe gait was observed in up to two males and two females from 2–48 h after dosing. A reduced splay reflex was seen in two females on day 2. Full recovery in all of these parameters occurred by 48 h after dosing.

At 50 mg/kg bw, qualitative clinical observations show slightly decreased/subdued activity in three males and two females on day 2, and tiptoe gait in two males and two females from 4 h after dosing. Reduced splay reflex was seen for one female on day 2 after treatment. On days 3 and 4, decreased activity was seen in one male only. Full recovery in all clinical parameters was apparent by 4 days after dosing.

At 20 mg/kg bw, slightly decreased activity was seen during clinical observation of one male from 0.5 to 1 h after dosing, and for three males and two females on day 2, and tiptoe gait was observed for two males and two females on day 2. A reduced splay reflex was seen for one female on day 2 of treatment. Full recovery of all signs was apparent by 2 days after dosing.

These reversible neuroactive effects were similar in severity and duration to those observed at 100 mg/kg in the preceding study in rats treated by gavage (Noakes, 2004b).

There were no toxicologically significant effects on body weight. Slightly low food consumption was seen for females treated with thiabendazole at 100 mg/kg bw on day 1 only.

Table 4. Results of a detailed functional observation battery (No. of animals affected) in a second study of acute toxicity in rats given thiabendazole by gavage

Parameters	Dose (mg/kg bw)							
	Males				Females			
	0	20	50	100	0	20	50	100
1–24 h								
Decreased activity	0	3	2	1	0	0	1	3
Reduced splay reflex	0	0	1	0	0	0	1	1
Reduced righting reflex	0	0	0	0	0	0	0	0
1-2 days								
Decreased activity	0	3	3	2	0	2	2	0
Reduced splay reflex	0	0	0	0	0	1	1	2
Reduced righting reflex	0	0	0	0	0	0	0	0
Day 15								
Decreased activity	0	0	0	0	0	0	0	0
Reduced splay reflex	0	0	0	0	0	0	1	0
Reduced righting reflex	0	0	1	0	0	0	0	0

From Noakes (2005a)

During the detailed FOB assessments, slightly decreased activity was observed 3 h after dosing for one male and three females at 100 mg/kg bw, two males and one female at 50 mg/kg bw, and three males at 20 mg/kg bw (Table 4).

A slightly reduced foot-withdrawal reflex was seen for one of 10 males from each of the groups at 20, 50 or 100 mg/kg bw and one of 10 females at 100 mg/kg bw. A slightly reduced splay reflex was also seen in one female at 100 mg/kg bw and in one male and one female at 50 mg/kg bw. Slightly decreased activity was still present 24 h after dosing for two males at 100 mg/kg bw, three males and two females at 50 mg/kg bw and three males and two females at 20 mg/kg bw. In contrast with the previous study (Noakes, 2004b), landing-foot splay was unaffected by treatment at any time. Overall motor activity was reduced for males at 100 mg/kg bw, 3 h after dosing. Slightly reduced motor

activity was most apparent for this group at 6–20 min and 26–30 min. There were no statistically significant differences in motor activity, in either sex, 24 h after dosing. No treatment-related adverse effects were present on day 15.

No treatment-related macroscopic abnormalities were observed during post-mortem examinations carried out at 24 h or 14 days.

In this repeat single-dose study of toxicity in rats treated by gavage, some minor and reversible clinical signs affecting motion characteristics were observed in all groups treated with thiabendazole, but generally at a low severity and incidence. Taking into account the overall response across this study and the preceding study, there was no obvious dose–response relationship in terms of numbers of animals affected or the severity of the findings. There did appear to be an effect of treatment on motor activity for males at 100 mg/kg bw, but this was only present at 3 h after dosing and the difference to controls was only relatively small. All these putative neuroactive effects were clearly reversible, with full recovery apparent by 14 days after dosing. In this study, there were no quantifiable differences in landing-foot splay for all groups of animals that received thiabendazole. The NOAEL was 100 mg/kg bw (Noakes, 2005a).

As an extension of these studies of toxicity with single doses administered by gavage and to confirm the NOAEL identified for the dietary route, groups of 10 male and 10 female CD®:Sprague-Dawley rats were fed diets containing thiabendazole (purity, 99.4%) at a concentration of 0, 300, 400, or 600 ppm for approximately 24 h. Achieved doses were equal to 0, 26, 34 and 48 mg/kg bw for males and 0, 26, 33 and 46 mg/kg bw for females. Feeding of experimental diets commenced immediately before the dark cycle on day –1 until the next dark cycle on day 1. Half the animals in each group were killed after 24 h and the rest were maintained on control diet for 14 days to assess the development of any findings. Clinical observations were recorded before feeding (day –1) then on day 1 at approximately 0.5 h (as part of an FOB), 2, 4 and 24 h (as part of an FOB) after the dark cycle ended on day 1, and at least once each day from days 3 to 14 (at approximately the same time) and on day 15 (as part of FOB). Body weights and food consumption were measured throughout the study. In addition, detailed clinical observations, including a qualitative assessment of sensory perception and quantitative assessments of landing foot splay, and assessment of motor activity were performed as soon as possible after the end of the dark cycle on day 1, to coincide with the time of peak effect of signs that could be related to peak plasma concentrations (T_{max}) measured in the study of kinetics after dietary administration (Duerden & Jones, 2005), 24 h after that (on day 2) and on day 15 before termination. After either 24 h or 14 days, the animals were killed and examined post mortem and specified tissues were taken for possible future histopathology examination. The study was performed according to the proposed test guideline document published by JMPR 2000, *Proposed test guideline – single-dose toxicity study by the oral route (for use in establishing acute reference doses for chemical residues in food and drinking water)*.[3] According to European Union[4] and JMPR guidance criteria, intake of test substance via dietary exposure is an acceptable dosing surrogate for gavage administration for the estimation of ARfD. The same conclusion can also be drawn based on results of comparative studies using administration by gavage or in the diet (Duerden & Jones, 2005).

[3] The document entitled *Guidance document for setting an acute reference dose (ARfD) (prepared by Germany)* from the European Commission, Directorate General for Agriculture VI B II.1, 7199/VI/99 rev. 3, dated 2 August 1999, was also consulted.

[4] *Opinion of the Scientific Committee on Plants on the draft guidance document for the setting of an acute reference dose (ARfD)* (SCP/GUIDE-ARFD/002-Final) from the European Commission Health & Consumer Protection Directorate General, dated 18 July 2002, Scientific Committee on Plants; Available from http://ec.europa.eu/food/fs/sc/scp/out133_ppp_en.pdf.

There were no treatment-related clinical signs at any dose. Reduced splay reflexes were observed among control and treated groups; however, the incidences and severity showed no relationship to treatment with thiabendazole and were considered to be incidental (Table 5).

Table 5. Detailed functional observation battery (No. of animals affected) in a study to establish an acute reference dose in rats given diets containing thiabendazole

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	300	400	600	0	300	400	600
1–24 h								
Decreased activity	0		0	0	0	0	0	0
Reduced splay reflex	0	2	0	1	4	4	2	2
Reduced righting reflex	0	+	0	0	0	0	0	0
1–2 days								
Decreased activity	0	0	0	0	0	0	0	0
Reduced splay reflex	0	3	1	0	2	3	2	3
Reduced righting reflex	0	0	0	0	0	0	0	0
Day 15								
Decreased activity	0	0	0	0	0	0	0	0
Reduced splay reflex	1	2	2	1	1	1	0	1
Reduced righting reflex	+	0	0	0	+	0	0	0

From Noakes (2005b)
+, qualitative effects.

FOB assessments and motor activity measurements did not reveal any treatment-related changes. Body weight and macroscopic findings showed no effects of treatment with thiabendazole. Although there appeared to be a slight reduction in food consumption for both males and females at 600 ppm for the 24 h during which the experimental diet was given; this was not accompanied by any associated reduction in body weight.

Clinical signs, FOB, motor activity and other investigations conducted in this study were initially performed shortly after the end of the dark cycle when plasma concentrations of thiabendazole were known to be at their highest level, based on the results from the preceding study of kinetics (Duerden & Jones, 2005). In view of the absence of treatment-related effects or clinical signs under this experimental scenario, the NOAEL for thiabendazole was at least 600 ppm (equal to 48 mg/kg bw for males and 46 mg/kg bw for females), the highest dose tested (Noakes, 2005b).

2.2 Reproductive toxicity

The following studies have previously been considered by JECFA and were reviewed by the present Meeting.

(a) Multigeneration studies

Rats

In a two-generation study of reproductive toxicity, groups of 33 male and 33 female Sprague-Dawley Crl:CD(SD)BR rats received diets containing thiabendazole (purity, > 99%) at concentrations providing a dose of 0, 10, 30 or 90 mg/kg bw per day. This study was summarized by JECFA in 1997 (JECFA, 1997). The only treatment-related findings were effects on food

consumption and body-weight gain in parental animals and offsprings, for which JECFA previously identified a NOAEL of 10 mg/kg bw per day. These effects were considered irrelevant for acute exposure. The NOAEL for reproduction was 90 mg/kg bw per day (the highest dose tested) based on a lack of any effect on reproductive performance (Wise & Lankas, 1992).

(b) Developmental toxicity

Mice

Three studies of developmental toxicity were undertaken in pregnant Jcl:ICR mice. Thiabendazole (purity, 98.5%) was given as a suspension in olive oil by gastric intubation. The animals were killed on day 18 of gestation. These studies were summarized by JECFA in 1992 (JECFA, 1993) and were reviewed by the present Meeting.

In the first experiment, mice were given thiabendazole at a dose of 0, 700, 1300 or 2400 mg/kg bw per day on days 7–15 of gestation. All fetuses were removed from the uterus on day 18 of gestation. Maternal body-weight gain was decreased in a dose-related fashion at all doses, and the mortality rate increased with increasing dose, being 0 out of 39 (controls), 0 out of 39 (lowest dose), 5 out of 39 (intermediate dose) and 24 out of 39 (highest dose). The weights of the liver, kidney, heart and spleen were increased at all three doses. A dose-related increase in the frequency of resorptions and a decrease in the number of live fetuses was seen at the two higher doses, while a dose-related decrease in fetal body weight occurred at all doses. In the offspring, the incidence of cleft palate was increased in a dose-related fashion at all doses, and the incidence of fusion of vertebral arches and vertebral bodies was increased in offspring of dams at the two lower doses. At the highest dose, only 20 fetuses from 3 litters were examined for external malformations and 15 fetuses from 2 litters for skeletal malformations (Ogata et al., 1984).

In the second experiment, animals were given thiabendazole at a dose of 2400 mg/kg bw on a single day between days 6 and 15 of gestation. All fetuses were removed from the uterus on day 18 of gestation. The maternal mortality rates were 2/7, 2/12, 1/12, 2/11, 2/11, 6/11, 2/11, 1/11, 4/11 and 6/11 on days 6–15 of gestation, respectively. The number of gestating females was markedly reduced in groups treated with thiabendazole on day 6 or 7 of gestation (1/7, 6/12, 9/12, 7/12, 9/11, 5/11, 9/11, 10/11, 7/11 and 4/11 on days 6–15, respectively). The rate of resorptions was increased and the fetal body weight decreased after dosing on any day. Increased frequencies of microcephaly and exencephaly were seen after treatment on day 6, 7 or 8; short or absent tail and anal atresia after dosing on day 9; open eyelids after treatment on day 7, 8, 10, 13 or 14; reduction deformity of the limbs after dosing on day 9, 10, 11 or 12; cleft palate after dosing on day 8, 9, 10, 11, 12 or 13; fusion of vertebral arches and vertebral bodies after treatment on day 7, 8, 9, 10 or 13; and fusion of the ribs after dosing on day 7, 8 or 9 (Table 6).

The reduction deformity of limbs observed was very significant (Ogata et al., 1984). This anomaly was not found in approximately 6000 fetuses of normal Jcl:ICR mice examined in this laboratory. In other laboratories, the spontaneous occurrence of this anomaly in this strain of mice was very low (0.02% amelia (absence of limb) and 0.02% oligodactilia among 5000 fetuses in one laboratory, and no deformities were seen in 4335 fetuses in another laboratory) (Kameyama, Tanimura & Yasuda, 1980).

Table 6. Incidence of malformations in fetuses of mice given thiabendazole at a dose of 2400 mg/kg bw orally on one of days 6–15 of gestation

Observation	Day of gestation									
	6	7	8	9	10	11	12	13	14	15
External malformations										
No. of litters with malformed fetuses/No. examined	1/1	3/6	2/9	4/7	4/8	3/4	2/7	7/10	1/6	0/2
No. of malformed fetuses/No. examined	1/15	6/64	3/108	11/58	5/92	23/41	3/81	28/125	1/79	0/24
No. of fetuses with:										
Microcephalia	1	0	0	0	0	0	0	0	0	0
Exencephalia	0	3	1	0	0	0	0	0	0	0
Reduction deformity of limbs	0	0	0	10	1	7	1	0	0	0
Short or absent tail	0	0	0	5	0	0	0	0	0	0
Anal atresia	0	0	0	4	0	0	0	00	0	0
Cleft palate	0	0	1	3	4	20	3	22	0	0
Open eyelids	0	5	1	0	2	0	0	6	1	0
Skeletal malformations										
No. of litters with malformed fetuses/No. examined	—	6/6	7/9	4/6	1/8	0/3	0/7	1/10	0/6	0/2
No. of malformed fetuses/No. examined	—	19/64	24/107	16/54	1/92	0/35	0/81	1/125	0/79	0/24
No of fetuses with:										
Fusion of vertebral arches	—	15	10	14	1	0	0	1	0	0
Fusion of vertebral bodies	—	1	1	1	0	0	0	0	0	0
Fusion of ribs	—	3	18	1	0	0	0	0	0	0

From Ogata et al. (1984)

In the third experiment, groups of 21–31 mice were given one of 17 different doses of thiabendazole at between 30 and 2400 mg/kg bw on day 9 of gestation. All fetuses were removed from the uterus on day 18 of gestation. Maternal body-weight gain was decreased at ≥ 1200 mg/kg bw, maternal mortality rate was increased at ≥ 1700 mg/kg bw (2/31, 3/31 and 7/31 females died at 1700, 2000 and 2400 mg/kg bw, respectively), and the weights of the liver, heart and kidney were decreased at ≥ 1400 mg/kg bw. The incidence of resorbed fetuses was increased at 1700 mg/kg bw, the mean number of live fetuses was significantly decreased at ≥ 2000 mg/kg bw and fetal body weight was decreased at ≥ 60 mg/kg bw. The incidences of external malformations such as cleft palate, exencephaly, open eyes lids and omphalocele (outpouching of the umbilicus containing internal organs) were similar in the control and treated groups. The incidence of reduction deformity of the limbs was increased at ≥ 480 mg/kg bw (statistically significantly from at 1200 mg/kg bw), and that of fusion of vertebral arches and vertebral bodies and of ribs was increased at ≥ 240 mg/kg bw (Tables 7 and 8) (Ogata et al., 1984).

Table 7. Incidence of external malformations in fetuses of mice given thiabendazole at a dose of 30–2400 mg/kg bw orally on day 9 of gestation

Dose (mg/kg bw) (grouped by experiment)	No. of litters with malformed fetuses/No. examined[a]	Percentage with malformation[b]	No. of malformed fetuses/No. examined	No. of fetuses with:						
				Reduction deformity of limbs[c]	Short or absent tail	Anal atresia	Cleft palate	Open eyelids	Exencephalia	Omphalocele
2400	9/18 (50.0)	24.6 ± 37.7*	16/179	9 (8; 44.4)***	6*[d]	0	0	0	0	0
2000	9/22 (40.9)	18.6 ± 34.2	14/189	7 (6; 27.3)***	2	0	2	7	2	0
1667	10/27 (37.0)	7.9 ± 13.7	17/305	7 (6; 22.2)**	8	0	0	5	0	0
1389	10/21 (47.6)	18.8 ± 31.1*	34/242	26 (6; 28.6)***	17*[d]	8	8	2	2	1
1127	4/20 (20.0)	6.5 ± 16.6	9/240	6 (3; 15.0)*	4	0	0	1	0	0
0	6/18 (33.3)	4.7 ± 9.7	11/246	0	0	0	2	6	3	0
1157	6/21 (28.6)	8.3 ± 22.9	8/213	2 (2; 9.5)	1	0	2	5	0	0
965	6/21 (28.6)	2.8 ± 4.7	7/251	0	0	0	0	7	1	0
804	4/22 (18.2)	1.9 ± 4.4	5/252	1 (1; 4.6)	0	0	0	4	0	0
670	4/19 (21.1)	3.5 ± 7.2	5/225	1 (1; 5.9)	0	0	0	4	0	0
558	4/22 (18.2)	1.8 ± 4.1	6/277	0	0	0	0	5	1	0
0	4/21 (19.0)	1.5 ± 3.3	4/244	0	0	0	2	1	1	0
558	3/21 (14.3)	1.9 ± 4.8	5/288	1 (1; 4.8)	0	0	0	4	0	0
269	3/23 (13.0)	1.5 ± 4.1	3/295	0	0	0	0	3	0	0
129	4/21 (19.0)	1.3 ± 2.8	4/294	0	0	0	0	3	1	0
62	4/22 (18.2)	2.2 ± 4.9	7/295	0	0	0	0	5	3	0
30	4/22 (18.2)	2.0 ± 4.9	6/300	0	0	0	0	6	0	0
0	3/20 (15.0)	0.7 ± 2.1	3/274	0	0	0	0	1	1	1
480	9/28 (32.1)	3.4 ± 6.3	13/365	2 (2; 7.1)	1	0	0	8	2	0
240	5/25 (20.0)	1.7 ± 3.9	5/362	0	0	0	0	5	0	0
120	6/27 (22.2)	2.3 ± 4.9	7/256	0	0	0	1	6	0	0

Dose (mg/kg bw) (grouped by experiment)	No. of litters with malformed fetuses/No. examined[a]	Percentage with malformation[b]	No. of malformed fetuses/No. examined	No. of fetuses with:						
				Reduction deformity of limbs[c]	Short or absent tail	Anal atresia	Cleft palate	Open eyelids	Exencephalia	Omphalocele
60	3/27 (11.1)	1.8 ± 6.1	5/361	0	0	0	3	2	1	0
30	4/28 (14.3)	1.2 ± 3.1	5/387	0	0	0	0	5	0	0
0	2/27 (7.4)	0.5 ± 1.9	2/347	0	0	0	0	2	0	0

From Ogata et al. (1984)

[a] Shown as a percentage in parentheses.

[b] Calculated by averaging the percentage in each litter (i.e. No. of malformations/No. of fetuses) and shown as mean ± standard deviation.

[c] In parentheses: No. of litters with affected fetuses; percentage of affected litters among those examined.

[d] The chi-squared test was used to compare the number of affected litters.

Values marked with asterisks are significantly different from those in the corresponding control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) as determined by the following statistical tests: chi-squared test (total incidence of litters with malformed fetuses and incidences of litters having fetuses with specific malformations); rank sum test (% incidence of malformations).

The paper by Ogata et al. (1984; see above) states that the time dependency for thiabendazole-induced malformations indicates that the compound is a non-specific teratogen. This time dependency is also characteristic of effects secondary to maternotoxicity. Cleft palate, exencephaly, open eye lids and vertebral anomalies are commonly observed in mice exposed to highly toxic doses of a variety of chemicals (Kavlock et al., 1985; Khera, 1984). Metabolic acidosis induced by carbon dioxide (CO_2) produces reduction limb deformity in mice (Weaver & Scott, 1984). Lack of controls (experiment 2) and precise data on maternal toxicity (only mortality was reported) compromise interpretation of the results of these three experiments. Excessive doses were used resulting in excessive maternal lethality and probably excessive toxicity (see further study in which decreases of 15 and 24% body-weight gain were observed in dams treated with thiabendazole at 100 or 200 mg/kg bw between days 6–15 of gestation). The number of fetuses/litters examined was sometimes very low, especially in the two first experiments. In the first two experiments, the authors reported the number of fetuses with specific malformations but not the number of litters affected. Therefore, the present Meeting considered the results of these three experiments as providing supplementary information only.

In a study of developmental toxicity, groups of 25 pregnant Jcl:ICR mice were given thiabendazole (purity, 99.8%) at a dose of 0, 25, 100 or 200 mg/kg bw per day in olive oil by oral gavage on days 6–15 of gestation. The animals were killed on day 18 of gestation. This study was evaluated by JECFA in 1997 (JECFA, 1997) and re-evaluated by the present Meeting. Dams at the two higher doses showed dose-related decreases in food consumption (13% below control levels on days 12–14 at the intermediate dose and 9–13% below control levels on days 6–8, 12–14, 14–16 and 16–18 at the highest dose) and body-weight gain (15% and 24% at the intermediate and highest dose, respectively), and there were dose-related decreases in the number of implantations (16.1, 15.4, 14.8 and 14.3 implants per pregnant female in the control group and at the lowest, intermediate and highest dose, respectively. The decrease was statistically significant ($p \leq 0.05$) at the two higher doses), dose-related decreases in the number of live fetuses (15.3, 14.6, 14.2 and 13.7, the decrease was statistically significant ($p \leq 0.05$) at the two higher doses) and statistically significant decreases in fetal body weight (97% and 95% of control values for females and 97% and 96% of control values for males at the intermediate and highest dose, respectively) at the two higher doses. An increased incidence of delayed ossification at a single site talus calcaneus was seen at all doses, but this was not dose-related, the number of affected litters was similar in all groups, including controls and was not considered to be a specific effect of thiabendazole on skeletal ossification. The NOAEL for maternal toxicity was 25 mg/kg bw per day on the basis of decreases in food consumption and body-weight gain at doses of 100 mg/kg bw per day or greater. The NOAEL for developmental toxicity was 25 mg/kg bw per day on the basis of the reduced number of implantations and live fetuses at doses of 100 mg/kg bw per day or greater (Nakatsuka et al., 1995).

Table 8. Incidence of skeletal malformations in fetuses of mice given thiabendazole at a dose of 30–2400 mg/kg bw orally on day 9 of gestation

Dose (mg/kg bw)	No. of litters with malformed fetuses/No. examined[a]	% malformed[b]	No; of malformed fetuses/No. examined	No. of fetuses with fusion of:		
				Vertebral arches	Vertebral bodies	Ribs
2400	12/18 (66.7)***	44.6 ± 42.3***	64/179	45	19	24
2000	17/21 (81.0)***	41.7 ± 39.0***	55/181	46	18	10
1667	18/27 (66.7)***	25.3 ± 28.2***	70/305	58	15	12
1389	13/21 (61.9)***	31.5 ± 39.1***	61/242	51	29	18
1127	11/19 (57.9)***	13.9 ± 17.9***	26/228	16	9	6
0	0/17 (0)	0	0/232	0	0	0

1157	12/21 (57.1)***	27.2 ± 33.9***	50/213	36	6	17
965	9/21 (42.9)*	11.6 ± 25.0***	27/251	19	9	5
804	9/22 (40.9)*	16.4 ± 23.6***	41/252	23	14	12
670	9/19 (47.4)**	15.2 ± 26.0***	36/225	34	4	5
558	9/22 (40.9)*	11.2 ± 16.9***	30/277	27	4	6
0	1/21 (4.8)	0.4 ± 1.8	1/244	0	1	0
558	7/21 (33.3)	8.1 ± 15.4*	22/288	18	1	5
269	4/23 (17.4)	1.5 ± 3.4	4/295	2	0	2
129	0/21 (0)	0	0/294	0	0	0
62	1/22 (4.5)	0.4 ± 1.6	1/295	1	1	1
30	0/22 (0)	0	0/300	0	0	0
0	1/20 (5.0)	0.4 ± 1.7	1/274	1	0	9
480	16/27 (59.3)***	16.7 ± 27.5***	52/352	46	0	7
240	7/25 (28.0)*	6.4 ± 16.3**	28/362	19	0	16
120	2/27 (7.4)	0.5 ± 2.0	2/256	0	1	1
60	2/27 (7.4)	0.7 ± 2.5	2/361	1	0	1
30	0/28 (0)	0	0/387	0	0	0
0	0/27 (0)	0	0/347	0	0	0

From Ogata et al. (1984)

[a] Shown as a percentage in parentheses.

[b] Calculated by averaging the percentage in each litter (i.e. No. of malformations/No. of fetuses) and shown as mean ± standard deviation.

Values marked with asterisks are significantly different from those in the corresponding control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) as determined by the following statistical tests: chi-squared test (total incidence of litters with malformed fetuses); rank sum test (% incidence of malformations).

Rats

In a study of developmental toxicity, groups of 25 pregnant Sprague-Dawley Crl:CD(SD) BR rats received thiabendazole (purity, 98.9%) at a daily dose of 0, 10, 40 or 80 mg/kg bw in 0.5% methylcellulose by gavage on days 6–17 of gestation. The animals were killed on day 20 of gestation. This study was evaluated by JECFA in 1992 (JECFA, 1993) and re-evaluated by the present Meeting. Food consumption and body-weight gain of the dams at the two higher doses were decreased in a dose-related manner (food consumption, 11–15% at the intermediate dose and 22–28% at the highest dose; body-weight, 2% at the intermediate dose and 26% at the highest dose), and dams at the highest dose showed ptosis (day 6 of gestation) and regurgitation. A dose-related decrease in fetal body weight was seen at the two higher doses (5% and 6% less than control values in females and 3% and 5% in males at the intermediate and highest dose, respectively). The NOAEL for maternal and developmental toxicity was 10 mg/kg bw per day (Wise, 1990; Lankas & Wise, 1993).

Rabbits

In a study of developmental toxicity, groups of 18 pregnant New Zealand White rabbits received thiabendazole (purity, 98.9%) at a dose of 0, 24, 120 or 600 mg/kg bw per day by gavage in 0.5% methylcellulose on days 6–18 of gestation. The animals were killed on day 29 of gestation. This study was evaluated by JECFA in 1992 (JECFA, 1993) and re-evaluated by the present Meeting. Dose-related decreases in food consumption (about 37–38% of control values on days 12–16 and 16–19)

and body-weight gain (decrease of 14% at 120 mg/kg bw per day) were seen at the two higher doses, with a loss of body weight at the highest dose (–230 g during the dosing period). Four out of 18 dams at the highest dose aborted. The mortality rates were 2 out of 18 controls (owing to intubation errors), 0 out of 18 at the lowest dose, 0 out of 18 at the intermediate dose and 1 out of 18 at the highest dose. A dose-related increase (not statistically evaluated) in the rate of early (mainly) and late resorptions was seen at the two higher doses (mean of 0.4, 0.6, 1.6 and 1.6 resorptions/litter, historical control litter average was 0.6, range: 0.2–1.0 resorptions). Fetuses at these doses (two fetuses from two litters at the highest dose and one fetus at the intermediate dose) also had dose-related increased incidences of domed head, hydrocephalus and marked enlargement of the fontanelle: fetal incidence, 0 out of 95, 0 out of 124, 1 out of 92 and 2 out of 56 (historical control incidence, 0.1%; range, 0–1.1%); litter incidence, 0 out of 14, 0 out of 16, 1 out of 14 and 2 out of 9 (historical control incidence, 0.7%; range, 0–8.3%). The NOAEL for maternal and developmental toxicity was 24 mg/kg bw per day (Hoberman, 1989; Lankas & Wise, 1993).

In another study of developmental toxicity, evaluated by JECFA in 1992 (JECFA, 1993) and re-evaluated by the present Meeting, groups of 18 pregnant New Zealand White rabbits received thiabendazole (purity, 98.6%) at a dose of 0, 50, 150 or 600 mg/kg bw per day in 0.5% methylcellulose by gavage on days 6–18 of gestation. The animals were killed on day 28 of gestation. Effects were seen only at the highest dose; they comprised decreased maternal food consumption (up to 20–30% less than control values on days 13–19) and weight gain (69% less than control value), increased rates of early and late resorptions (statistically significant increase in total resorptions, 11.8% compared with 8.1% in controls), decreased fetal body weight (11% and 13% less than control values in females and males, respectively) and increased incidences of common variation in lung lobation (11% compared with 1% in controls) and incompletely ossified metacarpals (24% compared with 5% in controls). No evidence for compound-related fetal hydrocephaly was found. The NOAEL for maternal and developmental toxicity was 150 mg/kg bw per day (Wise & Lankas, 1991 and Lankas & Wise, 1993).

3. Observations in humans

In a study in volunteers, 50 men aged 20–57 years received capsules containing placebo and 50 received 125 mg of thiabendazole twice per day for 24 weeks. Neither the study subjects nor the investigators were aware of who had received placebo. In total, 36 men receiving thiabendazole and 41 receiving placebo completed the study. One man was removed from the study at his own request because of daytime sedation and markedly decreased energy. The other withdrawals were clearly unrelated to the treatment. Weekly interviews were conducted to record side-effects. General physical examinations and laboratory examinations (haematology, measurement of cholesterol, glucose, urea, alkaline phosphatase, thymol turbidity, bilirubin in serum and urine analysis) were carried out before the test and after 4, 12 and 24 weeks. The haematological parameters measured were complete blood count including erythrocyte volume fraction. Protein-bound iodine in serum and electrocardiographic traces were evaluated only at the beginning and after 24 weeks of the study.

This study was previously summarized by the 1977 JMPR which noted that, under the conditions of the study, thiabendazole was well tolerated, and no effect on any of the parameters measured could be clearly ascribed to treatment. The JMPR identified a no-observed-effect level (NOEL) of 3–4 mg/kg bw per day, which was confirmed by JECFA in 1992 (JECFA, 1993). The following observations may be relevant for the acute toxicity of thiabendazole, although the time of onset of clinical signs was not specified. The men reported the following possible side-effects (treated

versus placebo): increased appetite (26 out of 50 vs 30 out of 50), flatulence (6 out of 50 vs 3 out of 50), nausea (4 out of 50 vs 2 out of 50), increased urinary frequency (3 out of 50 vs 3 out of 50) and sedation (7 out of 50 vs 5 out of 50) (Colmore, 1965).

In a review of studies, previously summarized by JECFA in 2002 (JECFA, 2002), on the efficacy of thiabendazole against parasites in humans, the standard therapeutic oral dose was 25 mg/kg bw twice per day for 1–4 days, although higher doses were used in some studies. The incidences of minor transient side-effects were generally 25–30% with the standard dose and higher with higher doses. The effects comprised anorexia, nausea, vomiting and dizziness. Serious side-effects were rare and comprised numbness, collapse, tinnitus, abnormal sensation in the eyes, xanthopsia, enuresis, decreased pulse rate and systolic blood pressure and transient rises in the frequency of cephalin flocculation (emulsions of cephalin are readily precipitated when mixed with serum of patients suffering from hepatitis and allied disorders of the liver, therefore this test is used for the recognition of certain hepatic diseases) and in aspartate aminotransferase activity (Campbell & Cuckler, 1969).

Side-effects in humans after therapeutic oral doses (not specified) of thiabendazole were reported in another literature review, previously summarized by JECFA in 2002 (JECFA, 2002). Common effects were dizziness (the frequency ranging from < 5% to 80%, depending on dosage) and nausea and vomiting (5–15%). Rarely observed side-effects included anorexia, abdominal pain, headache, drowsiness, weariness, heartburn, diarrhoea or constipation, flatulence, blurring of vision, xanthopsia, skin eruption, malodorous urine and vomiting of live *Ascaris*. The extent to which these frequencies differed from those in untreated subjects is unknown, although in two placebo-controlled studies, dizziness was reported to be approximately twice as common in thiabendazole-treated subjects as in placebo-treated subjects (Cuckler & Mezey, 1966).

In a clinical case report, previously summarized by JECFA in 2002 (JECFA, 2002), the following side-effects were reported in 14 of 23 patients with trichinosis who had received thiabendazole orally at a dose of 50 mg/kg bw as two daily doses for 10 days: nausea (11 out of 23), retching (11 out of 23), vomiting (11 out of 23), aversion to tablets (3 out of 23), exanthema (3 out of 23), impotence (2 out of 23), diarrhoea (1 out of 23), liver damage (1 out of 23), fever (1 out of 23) and dizziness (1 out of 23) (Hennekeuser et al., 1969). Again, the incidence of side-effects in untreated patients was not reported, and the extent to which these effects might have been influenced by the underlying condition was not reported.

From these surveillance data it is difficult to discern which of the effects are due solely to thiabendazole, or to patients' allergic or inflammatory responses resulting from the killing of microflorae, referred to as the Mazotti reaction, or to other possible non-drug effectors.

Comments

Previously evaluated studies

Studies to establish median lethal doses of thiabendazole given orally (LD_{50} values > 2000 mg/kg bw) did not provide any indication of acute effects.

In 2002, JECFA considered that emesis in dogs and effects on the kidney, haematopoietic system and development were relevant end-points for establishing an ARfD.

Clinical effects: In dogs, the NOAEL for emesis was 40 mg/kg bw per day. Common side-effects reported in humans receiving therapeutic doses (25 mg/kg bw or greater, twice per day for 1–10 days)

included anorexia, nausea, vomiting and dizziness. In a study in volunteers, in which controls were given a placebo, a dose of 125 mg of thiabendazole given twice per day for 24 weeks (equivalent to 3.6 mg/kg bw per day for a 60 kg person) did not cause significant changes in subjective side-effects.

Kidney effects: In single-dose studies in mice, renal toxicity, mainly in the proximal tubules, was observed at doses of 250 mg/kg bw and higher, and consisted of histopathological changes including mitochondrial swelling and ultimately necrosis of epithelial cells. Effects were most severe 2–3 days after dosing; after that time, tissue repair processes began. Apart from tubular dilatation, all effects were either fully or partly reversed within 10 days of administration. The NOAEL was 125 mg/kg bw.

Haematological effects: Changes indicative of anaemia were occasionally seen early in 4- or 13-week studies in rats and 14- and 53-week studies in dogs. As histopathological changes indicative of anaemia occurred after one or several doses, they were considered by the 2002 JECFA to be relevant for assessing acute exposure. The NOAELs for these effects in rats and dogs were 9 and 10 mg/kg bw per day, respectively. However, in single-dose studies in rats treated by gavage assessed by the present Meeting, no treatment-related changes in haematology parameters were observed at up to 1000 mg/kg bw, the highest dose tested.

In a study in volunteers, 50 men received an oral dose of 125 mg of thiabendazole twice per day for 24 weeks (equivalent to 3.6 mg/kg bw per day for a 60 kg person), and 50 other men were given a placebo. Thiabendazole did not affect haematological parameters in blood after 4, 12 or 24 weeks of treatment. However, histopathological examinations, which in animals were more sensitive indicators of haematotoxicity, were obviously not performed.

Developmental effects: As teratogenic effects and early resorptions may be induced by a single dose within a certain sensitive period, these effects on the fetus are particularly relevant to setting an ARfD. Five studies were provided for assessment by the present Meeting. In a published study in mice, teratogenic effects were observed after a single oral dose on day 9 of gestation. These effects consisted of deformed limbs at doses of 480 mg/kg bw and higher (NOAEL, 270 mg/kg bw) and fusion of vertebrae and ribs at 240 mg/kg bw and higher (NOAEL, 130 mg/kg bw). Excessive maternal mortality and lack of data on other maternally toxic effects compromised the interpretation of this study and the present Meeting considered this study as supplementary information only. In another study in mice, no teratogenic effects were observed when thiabendazole was given at doses of up to 200 mg/kg bw per day on days 6–15 of gestation. Thiabendazole was not teratogenic in rats at doses of up to 80 mg/kg bw per day, the highest dose tested.

Increased rates of resorption were observed in mice and rabbits, but not in rats. In mice, the NOAEL for this effect was 700 mg/kg bw per day when the animals were treated by gavage on days 7–15 of gestation, and 1400 mg/kg bw after a single dose administered by gavage on day 9 of gestation. Rabbits exposed on days 6–18 of gestation showed increased rates of resorption (mainly early resorption) at oral doses of 120 mg/kg bw per day and higher, with a NOAEL of 24 mg/kg bw per day. In another study in rabbits, rates of resorption were increased at 600 mg/kg bw per day, with a NOAEL of 150 mg/kg bw per day.

In another study in mice treated by gavage on days 6–15 of gestation, decreases in the number of implantations and in the number of live fetuses were observed at doses of 100 mg/kg bw per day and higher. The NOAEL was 25 mg/kg bw per day. The effects on implantation were considered to result from a direct effect of the substance since they were seen within the first few days after treatment, before maternal toxicity (decrease in food consumption and body weight) was observed.

Studies evaluated for the first time at this meeting

Single-dose studies of toxicity: Three single-dose studies of toxicity in rats were provided for assessment by the present Meeting. In the gavage studies, dose-related effects including reduced

activity, tiptoe gait, landing foot splay and reduced motor activity were observed for up to 24 h after 100 or 200 mg/kg bw and for up to 3 days after 1000 mg/kg bw. At this, the highest dose, there was also a transient reduction in body weight compared with controls. There were no treatment-related changes in haematology parameters. As the neuroactive effects observed at 100 mg/kg bw were marginal, the NOAEL was 100 mg/kg bw. In the dietary study, no treatment-related effects on clinical signs, FOB assessment, motor activity or body weight were observed at up to 600 ppm (equal to 46 mg/kg bw), the highest dose tested.

Toxicokinetic studies: Toxicokinetic studies that compared the gavage and dietary routes of administration showed that different kinetic profiles of thiabendazole were obtained, particularly with respect to C_{max}, which was shown to be much higher by the gavage route than by the dietary route. The administration of an aqueous slurry of diet as a model for residues of thiabendazole in food commodities containing a high residue of thiabendazole demonstrated that by this more relevant route of exposure the kinetic behaviour of thiabendazole was closer to the situation seen in the dietary study. Therefore the results of the dietary study would be more appropriate for deriving the ARfD. However, the substance was not tested at doses high enough to produce any toxic effects.

Toxicological evaluation

After considering the data available to the present Meeting as well as the 2002 JECFA evaluation, the Meeting established an ARfD of 0.3 mg/kg bw for women of childbearing age on the basis of the NOAEL of 25 mg/kg bw per day identified on the basis of reduction of implantations at doses of 100 mg/kg bw per day and higher in a study of developmental toxicity in mice, and a safety factor of 100. This value was supported by a NOAEL of 24 mg/kg bw per day identified on the basis of increases in resorptions at doses of 120 mg/kg bw per day and greater in a study of developmental toxicity in rabbits.

The Meeting established an ARfD of 1 mg/kg bw for the general population on the basis of a NOAEL of 100 mg/kg bw identified on the basis of some slight neuroactive effects at doses of 200 mg/kg bw and greater in a study of acute toxicity in rats treated by gavage, and a safety factor of 100.

Levels relevant to acute dietary risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Developmental toxicity[b]	Maternal toxicity	25 mg/kg bw per day	100 mg/kg bw per day
		Developmental toxicity	25 mg/kg bw per day	100 mg/kg bw per day
Rat	Single-dose toxicity study	Toxicity	100 mg/kg bwb	200 mg/kg bw
			600 ppm, equal to 46 mg/kg bw[a,c]	__
Rabbit	Developmental toxicity[b]	Maternal toxicity	24 mg/kg bw per day	120 mg/kg bw per day
		Developmental toxicity	24 mg/kg bw per day	120 mg/kg bw per day

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

Estimate of acute reference dose

0.3 mg/kg bw for women of childbearing age

1 mg/kg bw for the general population

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

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THIACLOPRID

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Explanation

Thiacloprid, (*Z*)-3-(6-chloro-3-pyridylmethyl)-1,3-thiazolidin-2-ylidenecyanamide (International Union of Pure and Applied Chemistry, IUPAC), is an insecticide of the neonicotinoid class. It acts as an agonist of the nicotinic acetylcholine receptor in the central nervous system, thus disturbing synaptic signal transmissions. Thiacloprid is an acute contact and stomach poison, with systemic properties. Thiacloprid was evaluated at the request of the Codex Committee on Pesticide residues (CCPR) and had not been evaluated previously by the Meeting.

All pivotal studies with thiacloprid were certified to be compliant with good laboratory practice (GLP).

Evaluation for acceptable daily intake

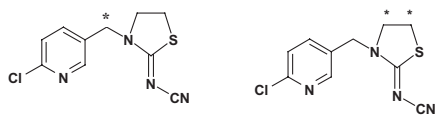
Unless otherwise stated, the studies evaluated in this monograph were performed by laboratories that were certified for GLP and complied with the relevant Organisation for Economic Co-operation and Development (OECD) test guideline(s) or similar guidelines of the European Union or United States Environmental Protection Agency (EPA). 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

The biokinetic behaviour and metabolism of thiacloprid in rats was investigated using the test compound labelled in the [methylene-^{14}C] position (Klein & Bornatsch, 1998) and in the [thiazolidine-^{14}C] position (Printz & Bornatsch, 1997). To describe the distribution kinetics of the total radioactivity in the various organs and tissues of the rat, a supplementary study of quantitative autoradiography was also conducted with [methylene-^{14}C]thiacloprid, using radioluminography technology (Klein, 1996). The respective positions of the radiolabel are shown in Figure 1.

Figure 1. Structure of thiacloprid and position of radiolabel



[^{14}C-methylene]thiacloprid [^{14}C-thiazolidine]thiacloprid

The investigations concerning toxicokinetics and metabolism in rats are summarized in this section. Since the study authors used different names or codes for the degradation products of thiacloprid in these studies, a single name and a single code number for each metabolite have been used in this summary. The structures, different names, short forms and code numbers of all animal metabolites as used in the study reports are also listed at the end of the monograph for cross-reference purposes (see Appendix 2).

1.1 Absorption, distribution and excretion

(a) ^{14}C-methylene labelled thiacloprid

In a study of absorption, distribution and excretion, groups of five male and five female Wistar rats received ^{14}C-methylene labelled thiacloprid (purity of unlabelled test substance, 99.6%; radiochemical purity, > 99%) at a single dose of 1 or 100 mg/kg bw in 0.5% tragacanth orally by gavage, or intravenously (i.v.) at a single dose at 1 mg/kg bw. A further group of five male and five female rats received unlabelled thiacloprid as 14 consecutive oral doses at 1 mg/kg bw per day followed by ^{14}C-labelled thiacloprid as a single oral dose at 1 mg/kg bw. For measurement of radioactivity in expired air, an additional test group of five male rats received a single oral dose at 1 mg/kg bw. Faeces, urine, plasma and expired carbon dioxide were collected for up to 48 h before the animals were sacrificed. A commercial computer program (TOPFIT; Thomae Optimized Pharmacokinetic Fitting Program, Version 2.0) was used to analyse the plasma curves and calculate the pharmacokinetic parameters. Linear standard compartment models (one to four compartments) were used for the computations.

Plasma concentrations of radioactivity indicated the rapid absorption of thiacloprid after oral administration (Table 1). Maximum plasma concentrations were achieved after 1–1.5 h at the lowest dose and 3–4 h at the highest dose. Maximum concentrations were similar in males and females of the groups at the lowest dose and receiving repeat doses. The maximum plasma concentration in groups of females receiving the highest dose was approximately 1.75 times that of the males. A comparison of the plasma curves suggested that absorption was slower and possibly incomplete at the highest dose.

In most of the tests, the elimination of radioactivity from plasma could be approximated by a combination of two exponential terms, from which elimination half-lives were calculated. The terminal half-lives varied between approximately 7 and 80 h. The final elimination phases took place at very low concentrations, hence their contribution to the area under the curve (AUC) was marginal. The AUC estimated for the groups at the highest dose, however, seemed to be unreliable and must be regarded as an artefact probably caused by an unsatisfactory mathematical description of the plasma curve with the model used. The large volume of distribution (Vd) suggests the rapid distribution of thiacloprid and/or its metabolites into tissues. The relatively small mean residence times (MRT) indicate that redistribution into plasma before excretion was also rapid. The report author states that the high MRT and $t_{½}$ values for females given repeated doses are due to an unsatisfactory mathematical model of the plasma curve. This assertion is supported by the comparable clearance (Cl) values for both sexes. The total clearance (average = 2.1 ml/min) had the same order of magnitude as the glomerular filtration rate of the rat.

Table 1. Plasma concentrations and kinetic parameters of ^{14}C-methylene-labelled thiacloprid in rats

Time-point	Mean plasma concentrations of radiolabelled thiacloprid (µg/kg equivalent)							
	1 mg/kg (iv)		1 mg/kg (gavage)		1 mg/kg (repeat doses, gavage)		100 mg/kg (gavage)	
	Male[d]	Female	Male	Female[d]	Male	Female[d]	Male	Female[d]
5 min	7.20	7.78	0.14	0.14	0.06	0.11	1.56	2.55
10 min	4.13	6.64	0.30	0.34	0.22	0.34	5.69	7.74
20 min	1.95	5.79	0.56	0.57	0.43	0.59	11.25	17.54
40 min	1.16	3.18	0.71	0.75	0.60	0.79	18.88	32.43
1 h	0.90	1.90	0.75	0.79	0.66	0.87	22.10	41.49
1.5 h	0.76	1.19	0.74	0.79	0.68	0.89	25.72	46.26
2 h	0.69	0.95	0.69	0.78	0.63	0.85	27.75	47.65
3 h	0.59	0.73	0.58	0.65	0.57	0.73	29.21	49.22
4 h	0.51	0.62	0.50	0.56	0.48	0.60	28.49	50.59
6 h	0.36	0.45	0.33	0.34	0.32	0.41	28.86	49.41
8 h	0.26	0.34	0.21	0.27	0.21	0.30	25.64	48.59
24 h	0.02	0.05	0.01	0.03	0.01	0.03	7.60	35.03
32 h	0.01	0.02	0.01	0.02	0.01	0.02	5.86	21.56
48 h	0.01	0.01	< 0.01	0.01	< 0.01	0.01	0.61	11.87
Kinetic parameters								
Vd (l/kg bw)	1.24	0.55	3.76	1.48	1.77	26.1	1.34	0.70
Cl (ml/min × kg bw)	1.80	1.30	3.00	2.87	3.07	1.83	1.85	0.99
$t_{½}$ (h)[a]	81.4	7.42	82.0	25.2	38.1	405.0[b]	9.15	12.3
AUC (µg/ml × h)	9.25	12.9	5.54	5.82	5.44	9.06	8.99[c]	16.8[c]
MRT (h)	11.5	7.1	21.7	9.8	11.1	238.0	15.2	25.3

From Klein & Bornatsch (1998)

AUC, area under the curve; Cl, clearance; MRT, mean residence times; Vd, volume of distribution.

[a] Terminal half-lives, mostly based on two exponential terms ([d] dose groups with four-compartment fit)

[b] The authors of the study report considered this result to be artificial because the unsatisfactory mathematical description of the plasma curve required three elimination terms.

[c] Result considered unreliable due an unsatisfactory mathematical description of the plasma curve with the model used

Radiolabel was excreted primarily in the urine (53.0–68.1%) and largely during the first 24 h after oral administration (Table 2). Significant faecal excretion of radioactivity was also seen in most groups (24.7–39.1%), with the exception of females at the highest dose (9.1%). This finding and the relatively high concentrations of radioactivity associated with the gastrointestinal tract in this group indicate delayed faecal excretion. The lower excretion rate in animals at the highest dose may be caused by to the slower absorption process. Faecal radioactivity in intravenously dosed animals was comparable to that in rats treated by gavage, suggesting significant biliary excretion of thiacloprid. Excretion of radiolabelled carbon dioxide in expired air was found to be minimal (≤ 0.005% of the administered dose).

Table 2. Excretion of radioactivity and radioactive residues of ^{14}C-methylene-labelled thiacloprid in rats

Sample	Time-point (h)	Excretion of radioactivity and radioactive residues (% of administered dose)								
		1 mg/kg (iv)		1 mg/kg (gavage)			1 mg/kg (repeat dose, gavage)		100 mg/kg (gavage)	
		Male	Female	Male	Male	Female	Male	Female	Male	Female
Urine	4	15.22	14.68	25.19	20.14	15.48	16.23	12.83	6.73	3.47
	8	38.09	31.66	42.06	45.83	32.67	38.27	26.39	20.15	7.39
	24	65.45	57.30	64.56	63.16	57.28	59.57	57.96	46.66	23.51
	48	68.11	61.30	66.79	64.70	60.25	61.28	60.12	65.52	52.97
Faeces	24	25.63	23.30	27.27	28.00	21.18	25.96	31.30	14.96	0.0
	48	29.29	27.48	30.94	30.11	24.70	29.60	34.01	39.11	9.12
Expired air	4	—	—	0.00	—	—	—	—	—	—
	8	—	—	0.00	—	—	—	—	—	—
	24	—	—	0.05	—	—	—	—	—	—
	48	—	—	0.05	—	—	—	—	—	—
Total excreted		97.41	88.79	97.79	94.82	84.94	84.94	94.13	104.63	62.10
Carcass		0.37	0.36	0.48	0.43	0.37	0.37	0.30	0.68	9.0
Gastrointestinal tract		0.28	0.20	0.18	0.10	0.12	0.12	0.11	0.95	17.6
Total recovery		98.1	89.3	98.4	95.4	85.4	85.4	94.5	106.3	88.7

From Klein & Bornatsch (1998)
iv, intravenous

Tissue residues at 48 h after dosing were minimal in rats at the lowest dose and in rats receiving repeated doses, accounting for 0.41–0.66% of the administered radioactivity (Table 3). Residues were slightly higher in males receiving the highest dose (1.63%), particularly in the liver and kidney. Total residues were significantly higher in females receiving the highest dose (26.6%), largely due to gastrointestinal residues. Findings indicated the delayed absorption or excretion of thiacloprid in this group.

Table 3. Residual radioactivity in organs and tissues of rats 48 h after dosing with ^{14}C-methylene labelled thiacloprid

Organ/tissue	Residual radioactivity (µg/g equivalent)							
	1 mg/kg (iv)		1 mg/kg (gavage)		1 mg/kg (repeated doses, gavage)		100 mg/kg (gavage)	
	Males	Females	Males	Females	Males	Females	Males	Females
Erythrocytes	0.0029	0.0035	0.0030	0.0036	0.0027	0.0032	0.4118	9.572
Plasma	0.0031	0.0042	0.0032	0.0041	0.0030	0.0035	0.4265	11.97
Spleen	0.0040	0.0032	0.0032	0.0031	0.0033	0.0032	0.3510	8.395
Gastrointestinal tract	0.0209	0.0193	0.0086	0.0140	0.0127	0.0116	5.5612	215.5
Liver	0.0147	0.0148	0.0165	0.0140	0.0178	0.0171	2.0905	30.23
Kidney	0.0093	0.0173	0.0109	0.0160	0.0136	0.0195	1.4715	23.04
Fat	0.0019	0.0033	0.0020	0.0016	0.0015	0.0016	0.2810	6.747
Testes	0.0022	—	0.0020	—	0.0017	—	0.2796	—
Uterus	—	0.0059	—	0.0029	—	0.0032	—	8.763
Muscle	0.0022	0.0022	0.0022	0.0025	0.0018	0.0020	0.2489	9.865
Bone	0.0024	0.0033	0.0022	0.0034	0.0026	0.0044	0.3112	5.223
Heart	0.0029	0.0031	0.0027	0.0034	0.0024	0.0031	0.4028	12.33
Lung	0.0057	0.0064	0.0065	0.0066	0.0047	0.0056	0.7116	13.15
Brain	0.0014	0.0019	0.0013	0.0018	0.0013	0.0018	0.1623	7.129
Skin	0.0041	0.0044	0.0060	0.0042	0.0032	0.0048	0.5190	10.07
Carcass	0.0025	0.0032	0.0035	0.0041	0.0022	0.0027	0.3095	10.47

From Klein & Bornatsch (1998)
iv, intravenous

[^{14}C-Methylene]thiacloprid was found to be rapidly and extensively absorbed in rats given a dose at 1.0 mg/kg. No significant differences in the extent or route of excretion were noted between dose groups or sexes, with the exception of females at the highest dose. Findings in this group were consistent with delayed absorption or faecal excretion. The results suggested that thiacloprid at a dose of 1 mg/kg bw is completely absorbed after oral administration in rats (Klein & Bornatsch, 1998).

In a study on the distribution of thiacloprid, male Wistar rats (one per time-point) were given a single dose of [^{14}C-methylene]thiacloprid (in 0.5% tragacanth) at 5.0 mg/kg bw by gavage. An additional rat was given a single intravenous dose at 1.0 mg/kg bw. The distribution of radioactivity was investigated at 5 min (intravenous dose) and at 1, 4, 8, 24 and 48 h (gavage dose) by whole-body autoradiography. Tissue concentrations of radioactivity were quantified using radioluminography.

Results indicated a rapid and even distribution of the radioactivity immediately (5 min) after intravenous injection. At this time, the concentration in the blood was already lower than in many of the organs, e.g. liver, kidney, muscle, preputial gland, adrenals, thyroid, salivary gland, and the walls of the aorta.

Thiacloprid was readily absorbed from the gastrointestinal tract, since radioactivity was found in all tissues and organs 1 h after oral administration. The general pattern of distribution observed 1 h after administering the parent compound was maintained throughout the investigation period of 48 h. Four hours after dosing, the radioactivity content of the stomach was still high, but faecal excretion had already commenced. The concentration of radioactivity in the kidney at 4 h and 8 h after dosing was consistent with the high proportion and rate of the renal excretion of the radioactivity. At later stages of the study, i.e. 24 h and 48 h after dosing, the most prominent feature, apart from

the excretory organs, was the radioactivity associated with the connective tissues in the skin, the aorta wall, and attached to the spinal cord, as well as the radioactivity still present in the glandular organs like thyroid, preputial gland, and adrenals. The low concentration in the adipose tissue at all times investigated reflected the fact that the parent compound and also its biotransformation products possessed a highly polar character.

Table 4. Equivalent concentration of the total radioactivity in tissues and organs of male rats given ^{14}C-methylene-labelled thiacloprid as oral or intravenous doses, as quantified by radioluminography

Sacrifice time (after dosing)	Concentration of radioactivity (µg/g fresh weight)					
	5 mg/kg (gavage)					1 mg/kg (iv)
	1 h	4 h	8 h	24 h	48 h	5 min
Blood	1.444	1.383	0.568	0.039	0.0034	0.422
Adrenal cortex	3.761	3.200	1.748	0.088	0.0049	1.112
Adrenal medulla	2.366	2.185	0.932	0.059	0.0033	0.760
Bone	2.734	1.992	0.617	0.065	< 0.0005	0.326
Bone marrow	1.621	1.587	0.683	0.040	< 0.0005	0.391
Brain	1.044	0.827	0.418	0.021	< 0.0005	0.352
Brown fat	1.660	1.453	0.811	0.053	< 0.0005	0.335
Connective tissue	2.771	2.605	1.306	0.152	0.0598	0.616
Heart	1.532	1.318	0.695	0.037	0.0009	0.532
Infraorbital gland	2.569	2.323	1.238	0.092	0.0172	0.756
Liver	4.243	3.732	2.088	0.169	0.0368	1.324
Lung	0.655	0.680	0.402	0.025	< 0.0005	0.278
Muscle	1.479	1.154	0.573	0.029	< 0.0005	0.503
Nasal mucosa	5.120	4.261	2.214	0.442	0.1617	0.622
Preputial gland	12.362	9.738	7.071	1.025	0.0288	1.029
Renal cortex	2.835	2.661	1.397	0.095	0.0218	0.735
Renal fat	3.374	1.918	1.174	0.044	0.0063	0.504
Renal medulla	2.215	2.260	1.016	0.056	0.0029	0.567
Salivary gland	1.786	1.418	0.760	0.046	< 0.0005	0.632
Skin	1.721	1.371	0.671	0.039	0.0017	0.408
Spleen	1.389	1.047	0.511	0.027	0.0006	0.415
Testes	0.737	0.629	0.314	0.020	< 0.0005	0.130
Thyroid	1.964	1.606	0.786	0.058	0.0237	0.631

From Klein (1996)

In the quantitative part of this study, the relative distribution pattern of the radioactivity was confirmed (Table 4). A steady decline in the equivalent concentration was observed for all organs from 1 h to 48 h after dosage. The values dropped from approximately 0.7 to 12 µg/g 1 h after oral administration to < 0.16 µg/g at the end of the test period (Klein, 1996).

(b) ^{14}C-thiazolidine labelled thiacloprid

In a study of absorption, distribution and excretion, groups of five male and five female Wistar rats (five of each sex per group) received ^{14}C-thiazolidine labelled thiacloprid (purity of unlabelled test substance, 99.6%; radiochemical purity, > 99%) as a single dose at 1 mg/kg bw in

0.5% tragacanth orally by gavage, while an additional group of five male rats received a single dose at 100 mg/kg bw. For measurement of radioactivity in expired air, a further test group of five male rats received a single oral dose of 1 mg/kg bw. Faeces, urine, plasma and expired carbon dioxide were collected for up to 48 h, when the animals were sacrificed. A commercial computer programme (TOPFIT) was used to analyse the plasma curves and calculate the pharmacokinetic parameters.

Plasma concentrations of radioactivity indicated the rapid absorption of thiacloprid (Table 5). Maximum plasma concentrations were attained at 2–3 h (lowest dose) and at 4 h (highest dose). Plasma concentrations of radioactivity also indicated the rapid distribution and elimination of thiacloprid at the lowest dose. Slower rates of absorption and excretion were seen at the highest dose, with significant plasma concentrations of radioactivity at 48 h. The large Vd suggested the rapid distribution of thiacloprid into tissues. The relatively small MRT indicated that redistribution into plasma before excretion was also rapid.

Table 5. Plasma concentrations and kinetic parameters of ^{14}C-thiazolidine-labelled thiacloprid in rats

Time-point	Mean plasma concentrations of radiolabelled thiacloprid (µg/kg equivalent)		
	1 mg/kg		100 mg/kg
	Males	Females	Males
5 min	0.11	0.07	1.46
10 min	0.23	0.19	5.75
20 min	0.43	0.35	13.07
40 min	0.57	0.51	25.38
1 h	0.64	0.58	32.94
1.5 h	0.65	0.64	35.49
2 h	0.66	0.66	32.34
3 h	0.58	0.69	39.04
4 h	0.55	0.68	50.34
6 h	0.42	0.59	33.07
8 h	0.32	0.48	34.84
24 h	0.07	0.07	23.16
32 h	0.05	0.04	25.15
48 h	0.03	0.02	10.38
Kinetic parameters			
Vd (ml/kg bw)	1450	1720	700
Cl (ml/min × kg bw)	1.82	1.60	1.07
$t_{½}$ (h)[a]	2.2/19.0	3.3/44.5	4.0/9.9
AUC (µg/ml × h)	9.19	10.4	1560
MRT (h)	16.5	22.8	25.3

From Printz & Bornatsch (1997)
AUC, area under the curve; Cl, clearance; MRT, mean residence times; Vd, volume of distribution.
[a] Half-lives for first elimination phase and for terminal elimination phase.

Radioactivity was excreted primarily in the urine (60.2–82.9%) and largely during the first 24 h after oral administration (Table 6). Faecal excretion was also significant (13.3–18.6%). Excretion of radiolabelled carbon dioxide in expired air was found to be minimal (0.86%).

Table 6. Excretion of radioactivity and radioactive residues of ^{14}C-thiazolidine-labelled thiacloprid in rats

Sample	Time-point	Excretion of radioactivity and radioactive residues (% of administered dose)			
		1 mg/kg bw			100 mg/kg
		Males	Males	Females	Males
Urine	4 h	3.87	6.64	9.62	4.64
	8 h	26.09	36.57	27.31	10.98
	24 h	66.31	71.31	77.01	32.18
	48 h	72.71	76.82	82.90	60.23
Faeces	24 h	15.73	11.95	8.47	0.98
	48 h	18.60	14.46	10.47	13.28
Expired air	48 h	0.86	—	—	—
Total excreted		91.51	91.28	93.37	73.51
Carcass		6.85	2.77	1.36	8.12
Gastrointestinal tract		0.45	0.40	0.21	4.25
Total recovery		99.68	94.45	94.94	85.88

From Printz & Bornatsch (1997)

Total tissue residues at 48 h in the group at the lowest dose accounted for 1.6–3.3% of the radioactivity administered (Table 7). Residues were highest in the liver in all groups at the lowest dose. In the group at the highest dose, residues accounted for 12.4% of the administered radioactivity, largely due to high residues in the gastrointestinal tract. Findings indicated delayed absorption or faecal excretion in this group.

Table 7. Residual radioactivity in organs and tissues of rats 48 h after dosing with ^{14}C-thiazolidine labelled thiacloprid

Organ/tissue	Residual radioactivity (µg/g equivalent)		
	1 mg/kg		100 mg/kg
	Males	Females	Males
Erythrocytes	0.021	0.015	7.612
Plasma	0.023	0.018	10.086
Spleen	0.032	0.021	10.329
Gastrointestinal tract	0.028	0.019	34.674
Liver	0.109	0.072	36.080
Kidney	0.063	0.046	19.484
Fat	0.001	0.005	6.306
Adrenals	0.061	0.040	29.561
Gonads	0.015	—	6.698
Uterus	—	0.019	—
Muscle	0.013	0.006	5.533
Bone	0.024	0.012	6.602
Heart	0.018	0.012	8.020
Lung	0.029	0.023	10.996
Brain	0.008	0.005	5.000

Thyroid	0.050	0.055	17.975
Skin	0.029	0.014	8.745
Carcass	0.017	0.009	6.540

From Printz & Bornatsch (1997)

^{14}C-thiazolidine-labelled thiacloprid was found to be rapidly and extensively absorbed in rats. No significant differences in the extent or route of excretion were noted between dose groups or sexes. Findings in the male group at the highest dose were consistent with delayed absorption (Printz & Bornatsch, 1997).

Plasma concentrations of radioactivity were comparable for ^{14}C-thiazolidine- and ^{14}C-methylene-labelled thiacloprid. The Cl values were slightly greater for [^{14}C-methylene]thiacloprid, while the AUC values were markedly greater for [^{14}C-thiazolidine]thiacloprid. The AUC value for [^{14}C-thiazolidine] thiacloprid was approximately 170 times greater than the value obtained for [^{14}C-methylene]thiacloprid at 100 mg/kg bw. However, the AUC estimated for the ^{14}C-thiazolidine-labelled thiacloprid at 100 mg/kg bw was considered to be unreliable due an unsatisfactory mathematical description of the plasma curve with the model used. Excretion of radioactivity in urine was slightly greater and consequently lower in faeces for ^{14}C-thiazolidine labelled thiacloprid. Residues of ^{14}C-thiazolidine-labelled thiacloprid were found to be higher than residues of ^{14}C-methylene-labelled thiacloprid.

1.2 Biotransformation

(a) ^{14}C-methylene-labelled thiacloprid

In a study of biotransformation, groups of five male and five female Wistar rats received ^{14}C-methylene-labelled thiacloprid (purity of unlabelled test substance, 99.6%; radiochemical purity, > 99%) as a single dose at 1 or 100 mg/kg bw in 0.5% tragacanth orally by gavage, or intravenously as a single dose at 1 mg/kg bw. An additional group received unlabelled thiacloprid as 14 consecutive oral doses at 1 mg/kg bw per day followed by radiolabelled thiacloprid as a single oral dose at 1 mg/kg bw. Faecal and urine samples analysed were collected and metabolites were identified by high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and proton nuclear magnetic resonance (^{1}H-NMR).

A total of 14 metabolites were identified, accounting for 57.8–78.7% of the administered radioactivity (Table 8). Small amounts (0.9–6.4%) of unchanged parent compound were also identified. With the exception of females at the highest dose, the major metabolite in all groups was identified as M07 (11.5–34.2%). The major metabolite in females at the highest dose was found to be (M12 + M13), accounting for 13.2% of the administered radioactivity. The major faecal metabolites were found to be M01 and unchanged parent compound. The amount of M11 was found to be significantly higher in males at the highest dose (13.4%) than in females at the highest dose (1.4%). Both M11 and M16 were found in higher amounts in male excreta while M06 and M07 were found in higher amounts in females.

The main metabolic transformation was the oxidative cleavage of the parent molecule to yield 6-chloronicotinic acid (M03) which then reacted with glycine to form a hippuric acid type conjugate (M07). Other metabolic reactions included oxidation of the *N*-nitrile group to form amide derivatives (M11 and M17), opening of the thiazolidine ring by oxidation to give sulfoxide compounds (M08 and M10) and the replacement of the chlorine atom attached to the pyridine ring of chloronicotinic acid by mercaptoacetic acid (M06). In the groups receiving the highest dose, the concentrations

of unchanged parent compound were increased while the concentrations of M07 decreased. This suggested that the metabolic rate was reduced at the highest dose (Klein & Bornatsch, 1998).

Table 8. Quantification of metabolites in the urine and faeces of rats 48 h after dosing with ^{14}C-methylene-labelled thiacloprid

Metabolite	Quantity of metabolite (% of recovered radioactivity)							
	1 mg/kg bw (iv)		1 mg/kg bw		1 mg/kg bw (repeated doses)		100 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine								
Thiacloprid	3.4	3.3	2.5	2.8	1.8	1.9	4.5	5.9
M01 (WAK 6856)	2.6	3.9	2.8	3.8	2.4	3.9	2.8	4.7
M03 (6-CNA)	6.8	9.7	7.1	8.7	8.0	9.1	9.0	4.2
M06 (KNO1886)	1.6	2.8	1.7	2.8	1.7	2.3	1.2	3.5
M07 (WAK 3583)	34.2	27.1	33.4	31.5	30.3	27.2	15.6	11.5
M08 (KNO 2672)	1.2	2.2	0.8	1.5	1.1	1.5	1.9	1.2
M09 (KNO1889)	2.4	6.1	3.3	5.1	3.6	5.6	1.4	1.1
M10 (KNO 1891)	1.4	1.2	1.4	1.7	1.2	0.9	ND	1.6
M11 (KNO 1893)	5.4	ND	3.6	ND	5.8	1.0	12.4	1.1
M12 (KNO 2621) + M13 (KNO 2665)	1.2	1.4	0.9	1.6	0.9	1.5	2.2	13.0
M14 (KNO 1872)	1.2	1.2	1.1	1.1	1.3	0.9	2.7	2.1
M15 (KNO 2684)	ND	0.5	ND	0.4	0.3	ND	0.8	1.7
M16 (NTN 35078)	2.9	1.3	2.8	1.1	2.4	0.6	4.0	1.1
M17 (KNO 1864)	1.0	0.7	0.7	0.8	0.7	0.4	0.4	0.6
Sum of metabolites in urine	65.4	61.3	62.1	62.8	61.6	56.9	59.1	53.2
Faeces								
Thiacloprid	2.9	2.6	2.1	1.5	2.0	1.8	6.4	0.9
M01 (WAK 6856)	3.2	1.6	2.8	1.4	3.1	1.9	5.8	1.4
M06 (KNO1886)	1.2	1.5	1.4	1.8	1.5	2.2	1.2	0.4
M08 (KNO 2672)	1.3	1.5	1.4	1.0	1.2	1.2	1.3	0.4
M09 (KNO1889)	0.3	0.7	0.6	0.4	0.5	0.4	0.3	0.2
M10 (KNO 1891)	0.4	ND	ND	ND	ND	0.4	ND	ND
M11 (KNO 1893)	1.2	0.5	1.1	0.7	1.0	0.8	1.0	0.3
M12 (KNO 2621) + M13 (KNO 2665)	0.7	0.6	0.6	0.5	0.4	0.4	0.9	0.2
M14 (KNO 1872)	0.4	0.7	0.6	0.4	0.5	0.5	0.7	0.2
M15 (KNO 2684)	ND	0.5	ND	ND	ND	0.9	0.3	0.2
M16 (NTN 35078)	0.8	0.3	1.0	0.3	0.8	1.0	1.2	0.2
M17 (KNO 1864)	0.3	0.2	0.2	0.2	0.3	0.3	0.4	0.1
Sum of metabolites in faeces	12.7	10.6	11.8	8.3	11.2	11.9	19.5	4.4
Total (urine + faeces)	78.0	72.0	74.0	71.2	72.6	68.8	78.7	57.8

From Klein & Bornatsch (1998)
iv, intravenous; ND, not detected.

(b) ^{14}C-thiazolidine-labelled thiacloprid

In a study of biotransformation, groups of five male and five female Wistar rats received ^{14}C-thiazolidine-labelled thiacloprid (purity of unlabelled test substance, 99.6%; radiochemical purity, > 99%) as a single dose at 1 mg/kg bw in 0.5% tragacanth orally by gavage, while an additional group of five male rats received a single dose at 100 mg/kg bw. Faecal and urine samples analysed were collected and metabolites were identified by HPLC, GC-MS, LC-MS and ^{1}H-NMR.

Thiacloprid was found to be extensively metabolized. A total of 17 metabolites accounting for 55.3–64.3% of the administered radioactivity were identified (Table 9). The major metabolites were found to be M19 (11.4%) in males at the lowest dose, M22 (22.2%) in females at the lowest dose and M01 (10.3%) in males at the highest dose. The major faecal metabolites were found to be M01 and unchanged parent compound.

The metabolic transformations included oxidative cleavage of the methylene bridge, hydroxylation and conjugation of the thiazolidine ring followed by further oxidation to the ketone, hydroxylation of the cyanamide moiety, opening of the thiazolidine ring at two positions followed by further oxidation to the carboxylic acid and methylation of the sulfur atom. The formation of an oxazole ring (M16) was explained by re-closure of the ring in the metabolite M26. The report stated that this reaction had been observed artificially for isolated M26 (Printz & Bornatsch, 1997).

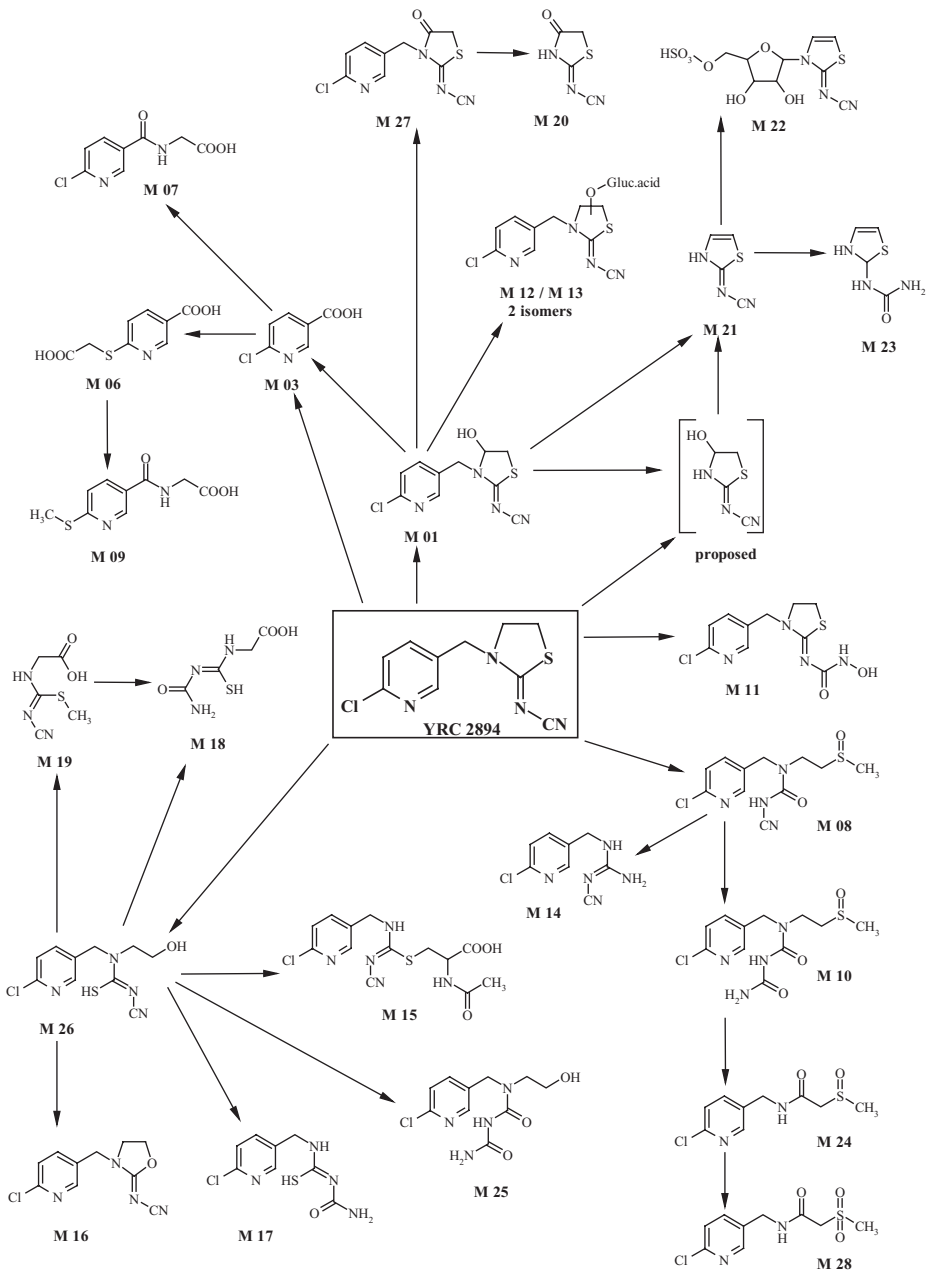
Table 9. Quantification of metabolites in urine and faeces of rats 48 h after dosing with ^{14}C-thiazolidine-labelled thiacloprid

Metabolite	Quantity of metabolite (% of recovered radioactivity)		
	1 mg/kg bw		100 mg/kg bw
	Males	Females	Males
Urine			
Thiacloprid	2.32	3.28	3.71
M01 (WAK 6856)	3.02	3.76	3.58
M08 (KNO 2672)	1.50	1.85	1.81
M10 (KNO 1891)	1.86	2.38	0.67
M11 (KNO 1893)	1.84	0.40	1.91
M12 (PIZ 1270), M13 (PIZ 1271)	2.03	1.19	6.52
M16 (NTN 35078)	2.67	1.12	5.60
M18 (PIZ 1241C)	6.14	3.91	0.84
M19 (PIZ 1250)	10.42	9.14	4.05
M20 (PIZ 1297B)	3.34	2.85	0.96
M21 (PIZ 1245)	0.61	1.29	0.62
M22 (PIZ 1243)	5.92	22.16	2.33
M23 (PIZ 1249)	5.43	5.23	1.60
M24 (PIZ 1297E)	ND	ND	0.88
M25 (PIZ 1297F)	ND	ND	0.62
M26 (PIZ 1253)	2.83	ND	5.73
M27 (PIZ 1297D)	0.99	0.74	2.53
Sum of metabolites in urine	50.93	59.30	43.97
Faeces			
Thiacloprid	2.21	2.22	3.10
M01 (WAK 6856)	3.22	1.63	6.72

M10 (KNO 1891) + M11 (KNO 1893)	0.35	0.20	0.47
M16 (NTN 35078)	0.51	0.16	0.61
M19 (PIZ 1250)	1.02	0.86	0.42
Sum of metabolites in faeces	7.31	5.06	11.31
Total (urine + faeces)	58.23	64.35	55.29

From Printz & Bornatsch (1997)
ND, not detected.

Figure 1. Proposed metabolic pathway of thiacloprid in rats



Note: For chemical names and codes see Appendix 2 at the end of this monograph.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

In a pilot study of acute oral toxicity, groups of five male and five female non-fasted Wistar (Hsd/Win:WU) rats were given thiacloprid (purity, 98.3%) as a single dose at 100–5000 mg/kg bw in demineralized water with 2% v/v Cremophor EL® by gavage.

Clinical signs occurred at all doses. The lowest lethal doses were 600 mg/kg bw in males and 370 mg/kg bw in females. Clinical signs started (depending on dose) between 59 min and 4 h after administration, and lasted up to 6 days. The main clinical signs were decreased motility and reactivity, poor reflexes, spastic gait, spasmodic state, convulsions, tremor, tachypnea, dyspnea, laboured breathing, diarrhoea, increased salivation, narrowed palpebral fissure, red incrusted margins of eyes. The median lethal dose (LD_{50}) was 621 mg/kg bw in males and 396 mg/kg bw in females (Kroetlinger, 1995a).

In a study of acute oral toxicity, groups of five male and five female fasted Wistar (Hsd Cpb:WU) rats were given thiacloprid (purity, 97.3%) as a single dose at 62.5–1000 mg/kg bw in demineralized water with 2% v/v Cremophor EL® by gavage.

At 62.5 mg/kg bw, no clinical signs occurred. The lowest doses causing symptoms were 300 mg/kg bw in males and 100 mg/kg bw in females. The lowest lethal doses were 700 mg/kg bw in males and 300 mg/kg bw in females. Clinical signs started (depending on dose) between 25 min and 6 h after administration, and lasted up to 5 days in males and up to 8 days in females. The main clinical signs were piloerection, constipation, decreased motility and reactivity, poor reflexes, spastic gait, spasmodic state, convulsions, tremor, tachypnea, dyspnea, laboured breathing, diarrhoea, increased salivation, narrowed palpebral fissure, closed eyelids, red excretion out of the nose and red incrusted snout. The LD_{50} was 836 mg/kg bw in males and 444 mg/kg bw in females. The oral dose causing no symptoms was 62.5 mg/kg bw in both sexes (Kroetlinger, 1996a).

In a study of acute dermal toxicity, groups of five male and five female Wistar (Hsd Cpb:WU) rats were given thiacloprid (purity, 97.3%) in 0.9% sodium chloride solution under an occlusive dressing to the clipped dorso-lumbar skin for 24 h. The treatment was tolerated by the animals without clinical signs, body-weight influences or mortalities. The acute dermal LD_{50} for thiacloprid in male and female rats was > 2000 mg/kg bw (Kroetlinger, 1996b).

In a study of acute inhalation toxicity, groups of five male and five female Wistar (Hsd/Win:WU) rats were exposed (directed-flow nose only) to a solid aerosol (dust) of thiacloprid (purity, 97.2%) at a concentration of 0, 80, 481, 1523 or 2535 mg/m^3 for 4 h. In addition to the clinical observations, body-weight measurements and gross necropsy, reflex tests (functional observational battery [FOB]) were conducted and the rectal temperatures were recorded on cessation of exposure. The aerosol generated was of adequate respirability at concentrations ≤ 481 mg/m^3 (i.e. mass median aerodynamic diameter, MMAD, approximately 3.1 µm; geometric standard deviation, GSD, approximately 1.7; aerosol mass < 3 µm approximately 49%). Because of technical difficulties with aerosol-generating equipment, the respirability of the aerosol particles was reduced at concentrations exceeding 500 mg/m^3 (i.e. MMAD, approximately 5.8–9.1 µm; GSD, approximately 2.4–3.5; aerosol mass, < 3 µm approximately 10–31%).

Concentrations of up to and including 481 mg/m^3 air did not induce compound-related mortality. Statistical comparisons between groups at 481–1523–2535 mg/m^3 air indicated significantly decreased body weights. Animals at the lowest dose tolerated the exposure without marked effects

on body weights. Exposure at 1523 and 2535 mg/m³ air produced mortality in females (from post-exposure days 1 to 7) but not in males. Clinical signs were observed at concentrations of 481 mg/m³ air and greater. Clinical signs consisted of concentration-dependent bradypnea, dyspnea, laboured breathing, rales, red encrustations around snout and nose, salivation, prostration, blepharospasm, mydriasis, chromodacryorrhoea, tremor, reduced motility, apathy, ungroomed hair, hypothermia, body-weight reductions, and piloerection. The duration of the observed clinical signs was dependent on respiratory signs and was up to 6 days. All animals showed normal reflexes, except some alteration in reflexes in the group at 1523 mg/m³. These changes were consistent with the clinical observations. The median lethal concentration (LC_{50}) was > 2535 mg/m³ air (2.535 mg/l air) in males and approximately 1223 mg/m³ air (1.223 mg/l air) in females (Pauluhn, 1996).

Table 10. Results of studies of acute oral, dermal and inhalation toxicity with thiacloprid

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg) LC_{50} (mg/m³)	Reference
Rat	Wistar (Hsd\Win:WU)	M & F	Oral (non-fasted)	98.3	M: 621 F: 396	Kroetlinger (1995a)
Rat	Wistar (Hsd Cpb:WU)	M & F	Oral (fasted)	97.3	M: 836 F: 444	Kroetlinger (1996a)
Rat	Wistar (Hsd Cpb:WU)	M & F	Dermal	97.3	M: > 2000 F: > 2000	Kroetlinger (1996b)
Rat	Wistar (Hsd/Win:WU)	M & F	Inhalation	97.2	M: > 2535 F: approximately 1223	Pauluhn (1996)

F, females; M, males.

(b) Dermal and ocular irritation and dermal sensitization

In a study on skin irritation potential, 500 mg of pulverized thiacloprid (purity, 97.3%) moistened with deionized water was applied to shorn dorso-lateral skin of three female New Zealand White (HC:NZW) rabbits under a semi-occluded dressing for 4 h. Skin reactions were scored on the Draize scale at 1, 24, 48, 72 and 168 h after treatment. Very slight erythema (grade 1) was observed in all three animals at 1 h and 24 h, but all the skin reactions had resolved by the 72 h. Therefore, the test substance is not a skin irritant (Kroetlinger, 1995b).

In a study on eye irritation potential, 100 µl (equivalent to 50 mg) of pulverized thiacloprid (purity, 97.3%) was instilled into the conjunctival sac of one eye of each of three New Zealand White (HC:NZW) rabbits. Ocular lesions were scored on the Draize scale at 1, 24, 48, 72 and 168 h after instillation. Twenty-four hours after instillation, the treated eyes were rinsed with normal saline. A 1% fluorescein solution was applied to the corneal surface to evaluate epithelial damage. No corneal or iridial lesions were evident. Conjunctival redness (grade 1) and swelling (grade 1 and 2) were seen in all three animals at 1 h and 24 h. All the ocular lesions had resolved by 48 h. Therefore, the test substance is not an eye irritant (Kroetlinger, 1995b).

The skin sensitization potential of thiacloprid (purity, 97.3%) suspended in physiological saline containing 2% Cremophor EL was investigated in female Dunkin-Hartley (Hsd Poc:DH) guinea pigs using a Magnusson & Kligman maximization test. In a range-finding study, intradermal injections of the test material (0.1 ml) at concentrations of 0–5% and topical induction applications of the test material (0.5 ml) at 0%, 12%, 25% or 50% did not induce skin reactions, while a concentration of 50% used for challenge produced slight localized erythema (grade 1) in two animals. The concentrations selected for the main study were 5% for intradermal induction, 50% for topical induction and 25% for challenge.

In the main study, one test group of ten animals and two control groups each consisting of five animals were used. The second control group was held in reserve incase a second challenge or further investigations were required. Each test animal received three pairs of the following intradermal induction injections (0.1 ml) into shorn skin in the scapular region: (i) Freund complete adjuvant diluted 1 : 1 with physiological saline solution; (ii) 5% thiacloprid in the vehicle; (iii) 5% thiacloprid formulated in the vehicle and Freund complete adjuvant (1 : 1). Six days after the injections, a topical application of 10% sodium lauryl sulfate in Vaseline was applied to the injection sites. Twenty-four hours later, a topical induction application of 50% thiacloprid (0.5 ml) was applied to the injection sites under an occlusive dressing for 48 h. The vehicle control animals received the same induction treatment without the test material.

Fourteen days after topical induction, both the test and one vehicle control group were given topical challenge applications to shorn flank skin for 24 h under an occlusive dressing. Each animal was given 25% thiacloprid (0.5 ml) on the left flank (caudal) and vehicle on the left flank (cranially). At the end of the exposure period, the application sites were cleaned with physiological saline. The skin reactions were scored at 48 h and 72 h after the start of the challenge applications (scale 0–3). Only 1 out of 10 test animals produced a positive response (grade 1) to the topical challenge applications. Although a positive control group was not included in this study, the sensitivity of the animal stock had been tested in contemporary studies. Therefore, thiacloprid is not a skin sensitizer (Stropp, 1996).

Table 11. Summary of irritation and skin sensitization potential of thiacloprid

Species	Strain	Sex	End-point (method)	Purity (%)	Result	Reference
Rabbit	HC:NZW	F	Skin irritation	97.3	Not irritating	Kroetlinger (1995b)
Rabbit	HC:NZW*	F	Eye irritation	97.3	Not irritating	Kroetlinger (1995b)
Guinea-pig	Dunkin-Hartley (Hsd Poc:DH)	F	Skin sensitization (Magnusson & Kligman)	97.3	Not sensitizing	Stropp (1996)

* NZW, New Zealand White.

2.2 Short-term studies of toxicity

Mice

In a pilot study, groups of three male and three female B6C3F_1 mice were fed diets containing thiacloprid (purity, 98.6%) at a concentration of 0, 100, 1000 or 10 000 ppm for up to 3 weeks. Clinical observations, body weight, feed and water intake were recorded at suitable time-points. A macroscopic examination was performed at necropsy and selected organs (liver and kidneys) were removed and weighed. The concentration, stability and homogeneity of the test material in the diet were acceptable. The daily intakes were equal to 0, 30.1, 367.8 and 4141.0 mg/kg bw per day in males, and 0, 63.9, 559.3 and 5785.1 mg/kg bw per day in females, at 0, 100, 1000 and 10 000 ppm, respectively.

No animals died during the study or showed clinical signs of toxicity. Body-weight gain was reduced in males at 10 000 ppm. At this dose, feed intake was increased and, therefore, feed efficiency was decreased in males. Feed intake was reduced in females at 1000 ppm and greater. Water intake was reduced in females at 10 000 ppm. Gross necropsy revealed enlarged livers in two out of three males at 10 000 ppm. Liver weights (absolute and relative) were increased in both sexes at 1000 ppm and greater.

The no-observed-adverse-effect level (NOAEL) was 100 ppm, equal to 30.1 and 63.9 mg/kg bw in males and females, respectively, on the basis of reduced feed intake in females and increased liver weight in both sexes at 1000 ppm (Wirnitzer, 1994).

Groups of five male and five female B6C3F_1/Bom mice were fed diets containing thiacloprid (purity, 98.6%) at a concentration of 0, 50, 200, 2000 or 10 000 ppm for 14 days. The objective of this study was to investigate the effects of the test material on the liver. Clinical signs of toxicity, body-weight changes, feed consumption and water intakes were recorded at suitable time-points. Haematological and biochemical investigations were carried out at the end of the study. All animals received a macroscopic examination at necropsy. The liver was removed, weighed and subjected to microscopic examination. Liver samples were taken for enzyme determinations. The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes were equal to 0, 21.6, 84.3, 765.1 and 4143.2 mg/kg bw per day in males, and 0, 29.8, 113.2, 1201.2 and 5449.8 mg/kg bw per day in females at doses of 0, 50, 200, 2000 and 10 000 ppm, respectively.

Table 12. Main findings of a 14-day study in mice fed diets containing thiacloprid

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	50	200	2000	10 000	0	50	200	2000	10 000
Body weight (g)	24	23	23	23	22	20	20	19	19	19
Liver weight (g)	1.36	1.28	1.41	1.56*	1.58**	1.05	1.06	1.06	1.23**	1.39**
Liver weight, relative (%)	5.67	5.48	6.03	6.71	7.08**	5.34	5.44	5.56	6.50**	7.41**
Hepatocytes, hypertrophy Average grade	0	0	41.3	42.0	53.0	0	11.0	0	31.3	52.4
Hepatocytes, fatty change Average grade	11.0	21.0	31.0	41.8	31.3	31.0	21.5	41.3	41.5	41.8
ECOD (nmol/g per min)	11.0	10.8	12.8	30.4	124.1	15.2	15.8	26.6	84.7	208.4
EROD (nmol/g per min)	0.50	0.56	0.75	0.90	3.53	0.57	0.50	0.71	2.43	2.99
ALD (nmol/g per min)	21.9	15.1	34.8	42.9	109.2	18.0	29.2	48.0	197.6	273.1
EH (nmol/g per min)	650	570	558	461	736	358	239	277	385	550
GST (µmol/g per min)	299.9	354.4	367.8	555.6	1 672.2	140.0	144.5	180.0	641.3	1 500.0
UDP-GT (nmol/g per min)	280	275	270	354	679	242	226	270	370	509

From Kroetlinger (1997)

* $p < 0.05$; ** $p < 0.01$.

ALD, aldrin epoxidase; ECOD, 7-ethoxycoumarin deethylase; EH, epoxide hydrolase; EROD, 7-ethoxyresorufin deethylase; GST, glutathione-*S*-transferase; UDP-GT, uridine diphosphate glucuronosyl transferase.

No animals died during the study or showed clinical signs of toxicity. Body weights and feed intakes were not affected by treatment. Water intakes were reduced in males at 2000 ppm and greater. In males at the highest dose, there was a significant reduction in the mean cholesterol value and a significant increase in the mean protein value, whereas in females, there was a significant reduction in mean albumin, cholesterol and bilirubin values at the highest dose. Dose-dependent enzyme induction was evident in the liver of both sexes at dietary concentraztions of ≥ 2000 ppm with some marginal effects at 200 ppm (Table 12). No gross findings were seen at necropsy. Mean liver weight (absolute and relative) was increased in both sexes at ≥ 2000 ppm (Table 12). Microscopy revealed hypertrophy of the centrilobular hepatocytes in males at ≥ 200 ppm and in females at ≥ 2000 ppm. The lipid content in hepatocytes was increased in both sexes at ≥ 2000 ppm.

The NOAEL was 200 ppm, equal to 84.3 and 113.2 mg/kg bw per day in males and females, respectively, on the basis of reduced water intake in males and liver effects (increased weight, increased lipid content of hepatocytes) at 2000 ppm and greater (Kroetlinger, 1997a).

Groups of 10 male and 10 female B6C3F_1 mice were fed diets containing thiacloprid (purity, 98.6–98.7%) at a concentration of 0, 50, 250, 1250 or 6250 ppm for about 14 weeks. Clinical signs of toxicity, body-weight changes, feed consumption and water intakes were recorded at suitable time-points. Haematological and biochemical investigations were carried out in weeks 12 and 13. All the animals received a macroscopic examination at necropsy. The following organs were removed and weighed: brain, liver, heart, spleen, kidneys, adrenals and gonads. Liver samples were taken for enzyme determinations. Sections of liver, kidney, pituitary gland, thyroid gland and organs or tissues exhibiting gross changes from all animals and dosage groups were examined microscopically. In addition, the testes and epididymides from animals dosed at 0 and 6250 ppm were examined. The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes were equal to 0, 19.9, 102.6, 542.4 and 2819.9 mg/kg bw per day in males, and 0, 27.2, 139.1, 704.3 and 3351.0 mg/kg bw per day in females at doses of 0, 50, 250, 1250 and 6250 ppm, respectively.

There were no treatment-induced clinical signs or mortalities during the study. At doses of 1250 and 6250 ppm, feed consumption in males seemed to be increased and feed efficiency was slightly reduced. At 6250 ppm males had significantly lower body weights (14%) and a reduced water intake. Haematological changes were not observed. Clinical chemistry revealed a decreased cholesterol content in females at ≥ 250 ppm and in males at 6250 ppm, and increased triglyceride concentrations at ≥ 6250 ppm in males. The bilirubin content was decreased at ≥ 1250 ppm in both sexes. At 6250 ppm, decreased protein content was detected in females. Liver enzyme induction (cytochrome P450 and *N*-demethylase) occurred in both sexes at 1250 and 6250 ppm, with marginal effects at 250 ppm.

Table 13. Main findings of a 14-week study in mice fed diets containing thiacloprid

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	50	250	1250	6250	0	50	250	1250	6250
Body weight (g)	30	28*	28	28	26**	24	24	24	24	24
Cholesterol (mmol/l)	3.02	3.06	2.92	2.79	2.10**	2.39	2.45	2.20*	2.20*	1.85**
Triglycerides (mmol/l)	1.76	1.74	1.88	2.14	2.84*	1.02	1.44	1.11	1.24	1.50
Bilirubin, total (µmol/l)	1.8	1.9	1.6	1.3**	0.9**	2.2	1.8	1.8	1.5**	1.2**
Protein (g/l)	54.1	54.5	55.7	55.4	55.3	56.0	53.2*	53.0*	53.7*	52.4**
Albumin (g/l)	30.2	29.0	31.0	30.9	29.9	34.1	33.0	33.8	33.1	31.1**
N-demethylase (mU/g)	109	117	146**	195**	230**	133	104*	138	195**	173*
P450 (nmol/g)	41	44	47	67**	103**	37	38	41*	59**	87**
Liver weight (g)	1.56	1.41**	1.40*	1.62	1.86**	1.22	1.19	1.25	1.32*	1.70**
Liver weight, relative (%)	5.21	5.03	5.08	5.69**	7.23**	5.09	5.04	5.10	5.59**	7.23**
Kidney weight (g)	0.54	0.49	0.48*	0.47*	0.43**	0.37	0.36	0.35	0.34*	0.35
Kidney weight, relative (%)	1.81	1.76	1.73	1.65*	1.68	1.53	1.50	1.44*	1.45*	1.50
Adrenal weight (mg)	9	6	6	6	8	12	12	15	18	17
Adrenal weight, relative. (%)	0.030	0.020	0.022	0.020	0.031	0.052	0.050	0.062	0.074	0.072

Hepatocytes, hypertrophy	0	2	6	9	10	0	0	1	10	10
Average grade	—	1.0	1.2	2.1	4.0	—	—	1.0	1.2	2.6
Adrenals, X zone, increased vacuolation	—	—	—	—	—	9	10	10	10	10
Grade 1 (minimal)	—	—	—	—	—	3	1	—	—	—
Grade 2 (slight)	—	—	—	—	—	6	3	—	—	—
Grade 3 (moderate)	—	—	—	—	—	—	6	6	—	—
Grade 4 (marked)	—	—	—	—	—	—	—	4	5	2
Grade 5 (massive)	—	—	—	—	—	—	—	—	5	8
Average grade	—	—	—	—	—	1.7	2.5	3.4	4.5	4.8

From Wirnitzer & Rühl-Fehlert (1995)
* $p < 0.05$; ** $p < 0.01$.

Liver weights were increased in both sexes at 1250 and 6250 ppm. Histopathologically, hypertrophy of hepatocytes was seen at doses of 250 ppm and greater in males, and of 1250 ppm and greater in females. The adrenal weights of females were slightly (but statistically not significantly) increased at ≥ 1250 ppm. This correlated with a dose-related increase in the severity of (fatty) vacuolation of the female adrenal X-zone at 50 ppm and greater leading to hypertrophy of this zone (Table 13). The X-zone is located between the zona reticularis and the adrenal medulla (function unclear). In mice, the X-zone involutes in males at puberty and persists in females until the first pregnancy. In non-pregnant females, it degenerates during adulthood depending on the genetic background of the animal (Shire & Beamer, 1983). In Swiss mice, it has been shown that the formation and degradation of this zone are influenced by pituitary and gonadal function (Deacon et al., 1986). The ovaries appeared to have lower numbers of old corpora lutea at 1250 and 6250 ppm. The interstitial glands of the ovaries appeared to be activated at 1250 ppm and greater. These glands are derived mostly from atretic follicles and respond to gonadotrophin stimulation but their function is unknown (Yuan & Foley, 2002). Kidney morphology and function were not affected in females and at doses of up to and including 250 ppm also not in males. At 1250 and 6250 ppm a reduction or loss of sex-specific vacuolation in the proximal tubules of kidneys was recorded in males.

The NOAEL was 50 ppm (equal to 19.9 and 27.2 mg/kg bw in males and females, respectively) on the basis of marked vacuolation of the adrenal X zone in females at 250 ppm and greater (Wirnitzer & Rühl-Fehlert, 1995).

Rats

In a pilot study, groups of three male and three female Wistar (Hsd/Win:WU) rats were given thiacloprid (purity, 98.3%) at a dose of 0, 5, 10, 20, 60 or 120 mg/kg bw per day in demineralized water with 2% v/v Cremophor EL by gavage for 14 days. The animals were regularly inspected and weighed; the feed and water intakes were determined by groups. Medical laboratory tests were carried out on blood and organ samples. Organs were weighed and subjected to gross pathological and histopathological examination. In addition to normal parameters, immunotoxicity investigations and determination of cell proliferation were also performed.

No animals died during the study. Clinical observations included reduced reactivity in males at 120 mg/kg bw per day, and reduced production of faeces in females at 60 mg/kg bw per day and greater. Mean body-weight gains and feed intakes were reduced in both sexes at 60 mg/kg bw per day and greater. There were no treatment-related effects on erythrocyte parameters but the mean leukocyte count was reduced in both sexes at 120 mg/kg bw per day. There were no treatment-related effects on thyroid hormone levels (thyroid-stimulating hormone [TSH], triiodothyronine [T3] and thyroxin [T4]).

Increased activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were noted in females at 120 mg/kg bw per day. Phase I enzymes in the liver were induced in both sexes at 60 and 120 mg/kg bw, while males also displayed a marginal enzyme induction at 20 mg/kg bw. Mainly cytochrome P450 and N/O demethylases, but also other monoxygenases and uridine diphosphate glucuronosyl transferase (UDP-GT) were involved (Table 14).

Table 14. Main findings in a 14-day study in rats given thiacloprid by gavage

Finding	Dose (mg/kg bw per day)											
	Males						Females					
	0	5	10	20	60	120	0	5	10	20	60	120
Body weight (g)	192	186	193	187	171	148	159	152	154	155	137	134
Feed intake (g/day):												
Day 7	19.1	18.3	18.2	17.7	13.9	10.3	15.5	15.2	15.9	15.9	11.7	10.1
Day 14	20.4	19.6	19.5	18.5	18.3	15.2	16.2	15.5	14.7	14.8	13.6	14.1
ASAT (U/l)	51.9	45.6	50.5	38.5	42.1	58.8	43.4	44.1	50.3	46.7	47.6	61.0
ALAT (U/l)	42.3	45.0	47.5	41.9	44.4	61.3	34.5	35.1	39.3	35.9	41.5	57
AP (U/l)	597	595	696	618	595	622	341	399	392	408	449	538
Cholesterol (mmol/l)	2.21	2.30	2.49	2.37	2.88	2.88	2.19	2.15	2.24	2.48	2.54	2.80
P450 (nmol/g)	33.8	33.6	40.8	43.6	49.9	70.5	32.1	30.3	27.5	26.5	40.8	55.0
O-DEM (mU/g)	9.5	11.0	14.2	14.9	23.2	39.2	8.1	7.4	7.9	8.7	14.4	29.8
N-DEM (mU/g)	149.4	155.4	187.9	229.1	279.8	427.3	67.7	73.4	65.4	72.1	125.2	253.2
Triglycerides (µmol/g)	5.78	5.45	5.41	5.87	5.61	6.16	5.33	5.29	6.73	5.86	5.60	6.80
ECOD (nmol/g per min)	5.9	9.9	9.7	12.4	16.6	37.6	2.9	2.8	3.2	3.6	5.1	21.7
EROD (nmol/g per min)	0.40	0.91	0.54	1.19	1.10	1.61	0.39	0.31	0.53	0.61	0.31	0.33
ALD (nmol/g per min)	90.2	163.9	101.6	138.4	241.2	274.8	18.9	13.8	19.7	17.8	27.3	60.7
EH (nmol/g per min)	353	559	467	517	982	1502	211	190	272	314	514	1011
GST (µmol/g per min)	87.0	88.9	84.3	96.3	132.4	174.1	87.0	90.8	92.6	90.7	137.0	187.9
UDP-GT (nmol/g per min)	283	297	355	380	639	797	133	151	163	169	304	529
Liver weight (g)	10.25	9.49	9.93	10.24	10.64	11.14	7.82	7.54	8.44	8.23	8.13	9.56
Liver weight, relative (%)	5.34	5.10	5.14	5.47	6.24	7.54	4.92	4.93	5.49	5.28	5.95	7.13
Thymus weight(g)	0.60	0.68	0.53	0.57	0.50	0.45	0.45	0.35	0.48	0.44	0.33	0.34
Thymus weight, relative (%)	0.31	0.36	0.27	0.31	0.29	0.31	0.28	0.23	0.31	0.28	0.24	0.26

From Kroetlinger (1995a)

ALAT, alanine aminotransferase; ALD, aldrin epoxidase; AP, alkaline phosphatase; ASAT, aspartate aminotransferase; ECOD, 7-ethoxycoumarin deethylase; EROD, 7-ethoxyresorufin deethylase; EH, epoxide hydrolase; GST, glutathione-*S*-transferase; N-DEM, *N*-demethylase; O-DEM, *O*-demethylase; UDP-GT, uridine diphosphate glucuronosyl transferase.

The immunotoxicological investigations revealed that the cell count in the spleen of males was reduced at 120 mg/kg bw per day, but this may have been a chance effect caused by large individual variations. Increased macrophage activation and other changes in cell counts were reported, but

they may also be related to variation and small sample size. The cell proliferation assays revealed increased cell proliferation in the perivenular region of the liver in females at 120 mg/kg bw per day. A reduction in cell proliferation, particularly in the renal medulla, and to a lesser extent in the renal cortex, was reported in females at 120 mg/kg bw per day.

Mean thymus weights (absolute) were reduced at ≥ 60 mg/kg bw per day. The mean relative liver weights of males and females were increased at ≥ 60 mg/kg bw per day, while mean absolute liver weight were increased in both sexes at 120 mg/kg bw per day. These liver weight changes correlated histopathologically with a slightly atypical structure of the hepatocellular cytoplasm (≥ 60 mg/kg bw per day in females, 120 mg/kg bw per day in males) and slight hepatocytic hypertrophy at 120 mg/kg bw per day in both sexes. Microscopy also revealed an increased mitotic rate in the thyroids of male rats at 120 mg/kg bw per day.

The NOAEL was 20 mg/kg bw per day on the basis of reduced body-weight gain and reduced feed intake at 60 mg/kg bw per day and greater (Kroetlinger, 1995a).

Groups of five male and five female Wistar (Hsd/Win:WU) rats were fed diets containing thiacloprid (purity, 98.6%) at a concentration of 0, 25, 100, 500 or 2000 ppm for 14 days. The objective of this study was to investigate the effects of the test material on the liver. Clinical signs of toxicity, body-weight changes, feed consumption and water intakes were recorded at suitable time-points. Clinical chemistry tests were carried out after 7 days and at the end of treatment. All the animals were examined macroscopically at termination. The liver and thyroid were removed and weighed, and subjected to gross and histopathological examinations. Liver samples were taken for enzyme determinations. The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes were equal to 0, 2.5, 11.2, 49.2 and 187.6 mg/kg bw per day in males, and 0, 2.3, 9.6, 49.5 and 187.2 mg/kg bw per day in females, at of 0, 25, 100, 500 and 2000 ppm, respectively.

No deaths occurred during the study. Constipation was evident on day 1 in one out of five females at 500 ppm and three out of five males and four out of five females at 2000 ppm. Significant reductions in mean body weight were seen in both sexes at 2000 ppm and small reductions were seen in females at 500 ppm. Feed consumption was significantly reduced during week 1 at 2000 ppm. Water intake was increased in females at 2000 ppm during week 2. Cholesterol levels were significantly increased in males at 100 ppm and greater and in females at 2000 ppm on day 7, while after 14 days of treatment, cholesterol levels were increased in both sexes at the highest dose only. There was a significant increase in TSH in females on day 14 at 2000 ppm. No treatment-related effects were seen on the levels of T3 and T4 or thyroxin-binding capacity. Liver enzymes (7-ethoxycoumarin deethylase [ECOD], aldrin epoxidase [ALD], epoxide hydrolase [EH], glutathione-*S*-transferase [GST], uridine diphosphate glucuronosyl transferase [UDP-GT]) were induced in both sexes at 500 ppm and greater (Table 15).

Table 15. Main findings of a 14-day study in rats fed diets containing thiacloprid

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	25	100	500	2000	0	25	100	500	2000
Body weight (g):										
Day 0	101	103	105	104	103	98	105	101	95	105
Day 7	148	148	151	139	115**	133	134	128	118*	111**
Day 14	196	196	194	184	147**	155	154	151	138	132**
GGT (U/l)	0	0	0	0	2*	0	0	0	0	2*
Cholesterol (mmol/l)	2.23	2.46	2.60	2.63	3.22**	2.17	2.28	2.39	2.43	3.37**

Bile acid (µmol/l)	45.9	31.7	53.6	48.2	136.3*	30.0	43.1	38.2	41.3	85.4
TSH (µg/l)	9.47	8.78	9.36	10.84	12.61	8.20	9.42	8.52	9.02	11.11*
ECOD (nmol/g per min)	7.3	7.5	8.3	21.0	48.7	3.1	2.3	4.1	7.6	25.4
ALD (nmol/g per min)	94.4	75.9	75.5	165.9	196.1	16.0	7.1	21.2	36.0	80.1
EH (nmol/g per min)	460	345	501	1062	2262	322	221	398	710	1486
GST (µmol/g per min)	86.1	85.0	98.9	169.4	262.2	85.6	82.2	96.1	137.8	251.7
UDP-GT (nmol/g per min)	499	513	604	887	1822	312	220	262	467	984
Liver weight (g)	10.09	9.76	9.65	10.24	10.43	7.62	7.31	7.56	7.40	8. 91*
Liver weight, relative (%)	5.14	4.98	4.97	5.57	7.09**	4.91	4.75	5.02	5.34	6.75**
Liver cell hypertrophy	0	0	0	5	5	0	0	0	3	5
Thyroid, hypertrophy follicular epithelium	0	0	0	0	5	0	0	0	0	1
Thyroid, increased mitotic index	1	1	1	4	5	0	2	0	2	2

From Kroetlinger (1996c)
* $p < 0.05$; ** $p < 0.01$;
ALD, aldrin epoxidase; EH, epoxide hydrolase; GGT, gamma-glutamyl transferase; GST, glutathione-*S*-transferase; ECOD, 7-ethoxycoumarin deethylase; TSH, thyroid-stimulating hormone; UDP-GT, uridine diphosphate glucuronosyl transferase.

Gross necropsy revealed liver distinct lobulation in males (four out of five) and females (two out of five) at 2000 ppm and in females at 25 (one out of five), 100 (one out of five) and 500 ppm (one out of five). There was an increase in the relative weights of liver (males and females) and thyroids (females) at 2000 ppm. Microscopic examination revealed hypertrophy of hepatocytes in both sexes at ≥ 500 ppm. This finding was combined with a "cytoplasmic change" showing a delicately structured cytoplasm. The thyroids of males exhibited an increased mitotic rate at ≥ 500 ppm and hypertrophy of the follicular epithelium at 2000 ppm.

The NOAEL was 100 ppm, equal to 11.2 and 9.8 mg/kg bw in males and females, respectively, on the basis of body-weight effects in females and thyroid effects (increased mitotic rate) in males at 500 ppm and greater (Kroetlinger, 1996c).

Groups of 10 male and 10 female Wistar (Hsd/Win:WU) rats were fed diets containing thiacloprid (purity, 98.6%) at a concentration of 0, 25, 100, 400 or 1600 ppm for up to 13 weeks. In addition, two recovery groups (10 of each sex per dose) received 0 or 1600 ppm for the same period of time and were observed for an additional 5 weeks. The study was performed in compliance with test guideline OECD 408. Furthermore, immunotoxicological measurements were included in this study. For this purpose, at the end of the treatment period blood (cardiac puncture), mesenteric lymph nodes, femur with bone marrow and half of the spleen of five animals per group were sampled and immediately chilled. The following parameters were determined: cell counts, FACScan analyses, macrophage activity after stimulation with phorbol 12-myristate 13-acetate (PMA), mitogen stimulation and antibody titres in serum. Furthermore, in this study liver and kidney samples were retained to check whether thiacloprid had an effect on proliferation in liver and kidney cells. The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes were 0, 1.9, 7.3, 28.6 and 123.2 mg/kg bw per day in males, and 0, 2.0, 7.6, 35.6 and 160.6 mg/kg bw per day in females at of 0, 25, 100, 400 or 1600 ppm, respectively.

No animals died during the study or showed clinical signs of toxicity. Mean body weights and body-weight gains were reduced in both sexes at 1600 ppm. At the end of the treatment period, body

weights of males were approximately 84% of the control value and of females were approximately 86% of the control value. During the recovery period, the body weights of the rats at 1600 ppm were lower than control values, but the difference diminished with time. Feed intakes were not affected by treatment, but water intake was reduced in males at 1600 ppm. No treatment-related ophthalmic findings were seen during this study.

The haematological examinations did not give evidence of adverse effects on erythrocytes or leukocytes. In females only, the clotting time was shortened after 1600 ppm. Clinical chemistry revealed a higher protein and an increased cholesterol concentration in males and females at ≥ 400 ppm. At 400 ppm, T3 concentrations were increased in males at week 3, but not at the end of the study at 100 and 400 ppm. At 1600 ppm, T3 concentrations and thyroid binding index (TBI) were increased in week 3 and 12 of the study. Induction of liver enzymes was detected at 400 ppm (cytochrome P450, N-DEM, O-DEM) and was more pronounced at 1600 ppm in both sexes (cytochrome P450, N-DEM, O-DEM, ECOD, ALD, EROD) (Table 16).

Thiacloprid had no effect on cell counts, or cell size distribution of spleen and lymph node cells. A slightly increased macrophage activation and increase of lipopolysaccharide (LPS) stimulated cells in the spleen was observed at 1600 ppm. No treatment-related changes of antibody titres for IgA, IgG and IgM were detected. Tests for a thiacloprid-mediated effect on cell proliferation in liver and kidney cells did not reveal evidence for a specific effect.

Relative liver weights were increased at 400 ppm in males and at 1600 ppm in both sexes. Thyroid weights were increased in males at the highest dose. Histopathologically, moderate hepatocellular hypertrophy, associated with a change of perinuclear cytoplasm (the cytoplasm exhibited a fine granular to vesicular structure), probably due to enzyme induction, was seen at 400 ppm and greater. At the end of the recovery period, only 3 out of 10 males had minimal hepatocellular hypertrophy, but cytoplasmic changes were no longer found (Table 16).

Table 16. Main findings of the 13-week study in rats fed diets containing thiacloprid

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	25	100	400	1600	0	25	100	400	1600
Body weight (g):										
Week 12	419	395	413	408	351**	220	228	236	209	190**
Week 17r	445	—	—	—	433	265	—	—	—	221**
Cholesterol (mmol/l):										
Week 3	2.47	2.48	2.49	2.77	3.37**	2.37	2.38	2.46	2.73**	3.71**
Week 12	2.38	2.29	2.49	2.65	3.25**	1.88	1.95	1.92	2.17**	3.45**
Week 17r	2.55	—	—	—	2.12**	2.50	—	—	—	2.18
Protein (g/l):										
Week 3	62.3	63.2	63.5	65.0*	65.5**	63.1	63.3	65.8*	66.1*	65.6*
Week 12	68.7	70.5	71.0	72.5**	76.5**	66.4	67.4	67.0	69.2	71.3**
Week 17r	72.4	—	—	—	72.5	71.0	—	—	—	68.9
T3 (nmol/l):										
Week 3	1.59	1.81**	1.87**	1.94**	2.26**	1.70	1.78	1.87	1.86	1.90
Week 12	1.62	1.61	1.58	1.71	2.11**	1.69	1.60	1.70	1.75	1.96
Week 17r	1.58	—	—	—	1.57	1.60	—	—	—	1.63
T4 (nmol/l), week 3										
Week 3	67	67	71	77**	75*	48	60	65*	57	48

Week 12	40	39	42	48	37	49	45	51	55	49
Week 17r	56	—	—	—	60	48	—	—	—	51
TBI:										
Week 3	0.79	0.79	0.79	0.80	0.84**	0.84	0.83	0.85	0.88*	0.89*
Week 12	0.88	0.93*	0.95*	0.94*	0.95*	0.84	0.88	0.83	0.85	0.82
Week 17r	0.76	—	—	—	0.80	0.80	—	—	—	0.84
N-DEM (mU/g):										
Week 12	105	95.5	113	180**	211**	76.4	69.8	65.7*	95.0**	153**
Week 17r	95.4	—	—	—	96.5	58.0	—	—	—	55.4
O-DEM (mU/g):										
Week 12	6.1	6.3	6.3	10.9**	30.4**	10.8	10.0	10.3	11.7	24.0**
Week 17r	10.4	—	—	—	11.6	11.4	—	—	—	9.1**
P 450 (nmol/g):										
Week 12	36.4	39.9	43.7*	62.8**	113**	33.3	35.8	34.2	40.8*	67.5**
Week 17r	39.5	—	—	—	40.8	31.6	—	—	—	31.2
Triglycerides (µmol/g):										
Week 12	9.44	8.80	8.82	9.04	10.03	6.01	5.95	6.17	6.38	6.37
Week 17r	6.24	—	—	—	5.83	6.04	—	—	—	5.90
ECOD (nmol/g per min):										
Week 12	5.2	4.9	5.0	8.7	37.2	4.2	3.9	4.4	7.3	21.1
Week 17r	6.0	—	—	—	5.7	3.1	—	—	—	3.2
EROD (nmol/g per min):										
Week 12	0.19	0.23	0.19	0.22	0.47	0.26	0.21	0.22	0.36	0.36
Week 17r	0.16	—	—	—	0.21	0.24	—	—	—	0.19
ALD (nmol/g per min):										
Week 12	76.4	67.0	72.1	104.6	300.4	21.4	23.5	21.7	39.1	89.4
Week 17r	11.9	—	—	—	94.5	14.4	—	—	—	13.6
EH (nmol/g per min):	412	404	408	595	1866	245	338	296	574	1660
Week 12	412	404	408	595	1866	245	338	296	574	1660
Week 17r	388	—	—	—	408	260	—	—	—	230
GST (µmol/g per min):										
Week 12	96.7	96.1	108.3	130.6	240.0	73.3	72.8	78.4	90.5	164.4
Week 17r	92.2	—	—	—	87.2	60.8	—	—	—	55.9
UDP-GT (nmol/g per min):										
Week 12	535	530	457	739	2826	347	409	405	662	1594
Week 17r	546	—	—	—	581	335	—	—	—	317
Liver weight (g):										
Week 12	14.4	13.1	13.6	15.1	17.4**	8.0	8.3	8.2	7.9	9.4**
Week 17r	14.2	—	—	—	14.5	8.0	—	—	—	7.9
Liver weight, relative (%):										
Week 12	3.36	3.27	3.25	3.61*	4.84**	3.46	3.49	3.34	3.65	4.77**
Week 17r	3.21	—	—	—	3.35	3.07	—	—	—	3.61**

Thyroid weight (mg):										
Week 12	6	8	9	9	10*	7	8	6	7	8
Week 17r	8	—	—	—	10	8	—	—	—	8
Thyroid weight, relative (mg/100 g bw)										
Week 12	1	2	2*	2	3**	3	3	2	3	4
Week 17r	2	—	—	—	2	3	—	—	—	4
Liver cell hypertrophy:										
Week 12	0	0	0	9	10	0	0	0	2	10
Week 17r	0	—	—	—	3	0	—	—	—	0
Liver cell cytoplasmic change, week 12	0	0	0	9	10	0	0	0	2	10

From Kroetlinger & Geiss (1997)

* $p < 0.05$; ** $p < 0.01$.

ALD, aldrin epoxidase; ECOD, 7-ethoxycoumarin deethylase; EH, epoxide hydrolase; EROD, 7-ethoxyresorufin deethylase; GST, glutathione-*S*-transferase; N-DEM, *N*-demethylase; O-DEM, *O*-demethylase; r, recovery group; T3, triiodothyronine TBI, thyroid binding index; UDP-GT, uridine diphosphate glucuronosyl transferase.

The NOAEL was established at 400 ppm (equal to 28.6 and 35.6 mg/kg bw in males and females, respectively) on the basis of reduced body weight, clinical chemistry changes (increased cholesterol and protein concentrations in plasma) and thyroid effects (increased weight) at 1600 ppm, the highest dose tested (Kroetlinger & Geiss, 1997).

In a pilot study on inhalation toxicity, groups of 10 male and 10 female Wistar (Hsd Win:WU) rats were exposed (nose only) to aerosolized thiacloprid (purity, 97.2%) at analytical concentrations of 0, 1.97, 19 or 205 mg/m³ air under dynamic conditions for 6 h per day on five consecutive days. The exposure period was followed by a recovery period of 3 days (interim sacrifice, 50% of the animals) or 2 weeks (terminal sacrifice). The MMADs (GSDs) of the particles were 3.3 µm (2.3), 2.9 µm (2.1) or 3.3 µm (1.8) at 1.97, 19 or 205 mg/m³ air, respectively. Between 44–55% of aerosol mass was less than 3 µm.

Clinical observations, body weight and feed intake were recorded at suitable time-points. Reflexes and the rectal temperatures were evaluated during the course of the study. Fifty per cent of the animals were sacrificed on the third post-exposure day. The remaining animals were sacrificed at the end of the 2-week observation period after exposure. All the animals received a macroscopic examination at necropsy. The following organs were removed and weighed: brain, kidneys, liver, thymus, thyroid, heart, lung, and spleen. Liver samples were taken for enzyme determinations. At each sacrifice, blood was sampled for basic haematological and biochemical determinations. Histopathology was not performed in this study.

No deaths occurred during the study. Clinical signs were observed at the highest dose and included ungroomed pelt, piloerection, reduced motility, tremor, bradypnea, laboured breathing pattern, flaccid appearance, mydriasis and emaciation. Based on the respiratory effects, the report concluded that the test aerosol had a minor potential to act as an upper respiratory tract irritant. All animals showed normal reflexes. An increase in grip strength was observed in both males and females at 19 mg/m³ and greater on day 4, but this finding was not confirmed by grip measurements made 3 days later. Rectal temperatures were significantly reduced at 205 mg/m³.

Significant reductions in body weight were observed in both sexes at 205 mg/m³ on day 4 (last exposure day) and day 7 (third day after exposure). Thereafter, the body weights of the control and treatment groups were similar. Feed intake was reduced in both sexes at 205 mg/m³ on day 4 (last exposure day).

At the highest dose, clotting time (hepatoquick) was decreased, protein levels were increased in males, and hepatic *N*-/*O*-demethylases, cytochrome P450, and liver triglycerides were significantly increased on day 7. Bile-acid concentrations and to a minor extent glutamate dehydrogenase activities were decreased, and hepatic *N*-/*O*-demethylases were statistically significantly increased in females on day 7. No biochemical changes were evident at the terminal sacrifice.

Gross necropsy revealed dark spleens at 19 mg/m³ (four females) and 205 mg/m³ (four females) at the interim necropsy. Dark spleens were not observed in any animals at the terminal necropsy. At 205 mg/m³, liver size and weight were increased, and thymus size and weight were reduced in males at the interim sacrifice. There were no treatment-related macroscopic findings or organ weight changes in any animals at the terminal sacrifice.

The no-observed-adverse-effect concentration (NOAEC) was 19 mg/m³ (0.019 mg/l air) for both sexes on the basis of clinical signs, reduced body weight, liver effects (increased weight, enzyme induction) and decreased thymus weights at 205 mg/m³. All effects had resolved by the end of the recovery period (Pauluhn, 1995).

In a study on toxicity by inhalation, groups of 10 male and 10 female Wistar (Hsd Win:WU) rats were exposed (nose only) to aerosolized thiacloprid (purity, 97.2%) at analytical concentrations of 0, 2.0, 18.2 or 143.4 mg/m³ air under dynamic conditions for 6 h per day on 5 days per week for 4 weeks. The group at the highest dose was initially exposed to a target concentration of 200 mg/m³ which led to marked respiratory distress and reduced body-weight gain so that it was reduced to 100 mg/m³ from the second week on. The study was performed in compliance with test guideline OECD 412. For all treatment groups, the MMAD of the particles was approximately 2.9 µm with a GSD of 1.8. Between 51–55% of aerosol mass was less than 3 µm.

Clinical observations, body weight and feed intake were recorded at suitable time-points. Reflexes and the rectal temperatures were evaluated during the course of the study. Ophthalmological examinations were performed before the start of the study and towards the end. Urine analysis was also performed near to the end of the study. All the animals were given a macroscopic examination at necropsy. Selected organs were removed and weighed, and blood was sampled for haematological and biochemical determinations. Histopathology of retained organs and tissues was performed in this study.

No deaths occurred during the study. Clinical signs were observed at the highest dose and included bradypnea, reduced motility, tremor, laboured breathing pattern, piloerection, ungroomed hair-coat, atony, rales, salivation, mydriasis, miosis and vocalization. These signs were considered to be a consequence of respiratory distress rather than as central nervous effects. Reduced body weights (days 4–11) and hypothermia were evident in both sexes at the highest dose. No treatment-related effects were revealed by the ophthalmological examinations.

Erythrocyte parameters were not affected by treatment. There were significant increases in the concentrations of phosphate, glucose, cholesterol, bile acids and calcium and increased alkaline phosphatase activity in females at the highest dose. Glucose and phosphate concentrations were significantly increased in males at the highest dose. Liver enzyme induction was detected at 143.4 mg/m³ air and was more pronounced in females than in males. A marginal effect in some enzymes was also seen at 18.2 mg/m³ (Table 17). Changes indicative of an effect on the thyroid were not seen during this study. Urine analysis did not reveal any treatment-related effects.

Table 17. Relevant findings in a 4-week study in rats exposed to thiacloprid by inhalation

Finding	Dose (mg/m³)							
	Males				Females			
	0	2	18.2	143.4	0	2	18.2	143.4
Body weight (g):								
Day 4	242	240	237	217**	166	166	167	151**
Day 28	295	287	292	290	187	186	189	192
Cholesterol (mmol/l)	1.70	1.68	1.64	1.86	1.62	1.65	1.74	2.12**
AP (U/l)	295	292	310	339	189	206	198	226*
Bile acids (µmol/l)	23.0	25.3	27.4	25.2	29.8	21.4	20.1	42.6*
N-DEM (mU/g)	122	135	149*	176**	67	52*	65	144**
O-DEM (mU/g)	9.8	11.1	11.4	18.5**	8.8	8.3	8.5	18.0**
P 450 (nmol/g)	41.8	41.6	44.6	57.4**	34.3	33.5	36.6	53.6**
Liver weight (g)	11.06	10.87	11.01	12.21	7.19	7.10	7.22	8.40**
Relative liver weight (g/100g)	3.75	3.79	3.78	4.21**	3.90	3.86	3.86	4.44**
Thyroid weight (mg)	6.2	7.1	9.6	8.5	5.8	4.9	5.9	7.7
Relative thyroid weight (mg/100g)	2.1	2.5	3.3	2.9	3.1	2.7	3.1	4.0
Liver, hepatocyte hypertrophy	0	0	5	7	0	0	0	4
Thyroid, follicular epithelium hypertrophy.	0	0	0	2	0	0	0	0

From Pauluhn (1998)

* $p < 0.05$; ** $p < 0.01$

AP, alkaline phosphatase; O-DEM, *O*-demethylase; N-DEM, *N*-demethylase.

There were no treatment-related macroscopic findings at necropsy. The main organ weight changes are presented in Table 17. The increased lung weights in males lacked a dose–response relationship and there was no compound-induced effect on female lung weight. Therefore, the lung-weight changes in males were considered to be incidental. The thyroid weights of males were slightly increased at 18.2 mg/m³ and 143.4 mg/m³ (not in a dose-dependent amnner).

The microscopic examination revealed minimal to slight hypertrophy of hepatocytes at ≥ 18.2 mg/m³ air in males and at 143.4 mg/m³ in females. Slight hypertrophy was observed in the thyroidal follicular epithelium of two males at 143.4 mg/m³. Microscopy did not detect any treatment-related findings in the respiratory tract.

The NOAEC was 18.2 mg/m³ (0.018 mg/l air) on the basis of clinical signs, reduced body weights, liver toxicity in females (increased plasma concentrations of cholesterol, alkaline phosphatase and bile acids) and thyroid effects in males (increased weight, hypertrophy of follicular epithelium) at 143.4 mg/m³ (Pauluhn, 1998).

In a short-term study of dermal toxicity, groups of five male and five female Wistar (Hsd Cpb:WU) rats were given thiacloprid (purity, 97.2 %) at a dose of 0, 100, 300 or 1000 mg/kg bw per day by dermal application. The animals were exposed for 6 h per day and received 22 applications for 28 days. Additionally, satellite groups of five males and five females were given a dose at 0 or 1000 mg/kg bw per day followed by a 2 week recovery period. The test substance was applied undiluted and moistened with water immediately before application. After exposure, the dressings were removed and the application sites were cleaned with soap and water. The study was performed in compliance with test guideline OECD 410. Dose selection was based on a 3-week dose range-finding study using doses of 0 and 1000 mg/kg bw per day. The effects reported in this study included lower feed intake, leukocyte changes, increased cholesterol levels,

reduced triglyceride concentrations, increased liver weight, lower thymus weights and dark-red spleens.

Clinical observations, body weight and feed intake were recorded at suitable time-points. The treated skin sites were examined daily and any reactions were scored on the Draize scale. All the animals were given a macroscopic examination at necropsy. Blood samples were taken for haematological and biochemical determinations. The following organs were removed and weighed: brain, heart, lungs, liver, spleen, kidneys, adrenals, thymus and testes. Histopathology of retained organs and tissues was carried out.

No animals died during the study or showed clinical signs of toxicity. There were no treatment-related effects on body weight. No local skin reactions were observed at the treatment sites. In females, there was a transient decrease in feed intake at 1000 mg/kg bw per day. The haematological and clinical chemistry examinations did not reveal any treatment-related changes. There were no treatment-related macroscopic findings at necropsy. The mean liver weights (absolute and relative) were increased in both sexes at 1000 mg/kg bw per day (Table 18). At the end of the recovery period, the liver weights of the group at 1000 mg/kg bw per day were comparable to control values. Microscopy revealed centrilobular hypertrophy in combination with a more homogeneously structured cytoplasm in male livers at doses of ≥ 300 mg/kg bw per day and in females at 1000 mg/kg bw per day. These findings were considered to be a consequence of liver enzyme induction. At the end of the recovery period, these changes were still evident in two out of five males but not in females. The thyroids of males and females displayed follicular cell hypertrophy at 1000 mg/kg bw. This effect was reversible in females, but not completely reversible in males within the 2-week recovery period (one out of five males still exhibited the effect). There were no treatment-related microscopic skin findings at the treatment sites.

Table 18. Relevant findings in a 4-week study of dermal toxicity in rats given thiacloprid by dermal application

Finding	Dose (mg/kg bw per day)							
	Males				Females			
	0	100	300	1000	0	100	300	1000
Body weight (g)	306	299	286	296	218	221	217	221
Liver weight (g)	12.65	12.7	12.23	14.26	8.70	8.87	9.12	9.88**
Liver weight, relative (g/100g)	4.10	4.25	4.26	4.81**	3.99	4.01	4.21	4.47*
Liver, hepatocyte hypertrophy	0	1	3	5	0	0	0	3
Thyroid, follicular. epithelium hypertrophy.	0	0	0	3	0	0	0	2
Recovery groups								
Body weight (g)	336	—	—	321	231	—	—	230
Liver weight (g)	13.45	—	—	12.84	8.97	—	—	9.14
Relative weight (g/100g)	4.01	—	—	4.00	3.89	—	—	3.97
Liver, hepatocyte hypertrophy	0	—	—	2	0	—	—	0
Thyroid, follicular. epithelium hypertrophy	0	—	—	1	0	—	—	0

From Kroetlinger & Sander (1997)

* $p < 0.05$; ** $p < 0.01$

The NOAEL for systemic toxicity was 300 mg/kg bw per day in males and females on the basis of thyroid follicular cell hypertrophy at 1000 mg/kg bw per day. Hypertrophy of hepatocytes and thyroid follicular cells in males at 1000 mg/kg bw per day was partially reversible after a 2-week recovery period. The NOAEL for skin reactions was 1000 mg/kg bw per day, the highest dose tested (Kroetlinger, 1997b).

Dogs

In a dose range-finding study, groups of two male and two female beagle dogs were given diets containing thiacloprid (purity, 98.6%) at a concentration of 0, 100, 300, or 1000 ppm for up to 10 weeks. Since no clinical signs and no other effects were seen, the highest dose was increased from 1000 to 1250 ppm from day 19 of treatment onwards, and, as no effects occurred, to 1600 ppm from day 26 of treatment onwards. This dose was further increased to 2500 ppm on day 38. This dose was administered until the end of the study. A satellite group of two male and two females was given a dietary concentration of 2500 ppm from the beginning of the study for 4 weeks. The doses in study week 9 were equal to average doses of 0, 3.3, 9.6, 80.0 and 65.7 mg/kg bw per day (satellite group) for both sexes.

In the group at 1000 ppm, a slight reduction in feed intake and body-weight gain was observed, starting after the dose increase with week 4, while after a 4-week administration of 2500 ppm to a separate group of dogs, reduced feed intakes and reduced body-weight gains were detected. Other symptoms were not observed. In the groups at 1000 and 2500 ppm, slightly increased urea and creatinine concentrations were detected. In males treated with thiacloprid at 2500 ppm from the beginning of the study, slightly increased alanine transferase activity was seen which was partly reversible during the study period. Slight changes in the thyroid hormones were observed: T4 was slightly reduced and T3 and thyroxin-binding capacity were slightly increased in females at 2500 ppm at study initiation. Marginally increased activities of liver enzymes, ECOD and EH and, in females only, of GST were seen in the animals treated at 2500 ppm at study initiation. Urine analysis did not reveal any changes. No gross findings were detected. Increased prostate weights were found in males at 1000 and 2500 ppm. Histopathologically, slight cytoplasmic changes in the liver were detected in one male and one female treated at 2500 ppm for 4 weeks from study initiation, and in one female treated initially with a dose of 1000 ppm which was gradually increased to 2500 ppm.

The NOAEL was 300 ppm, equal to 9.6 mg/kg bw, on the basis of reduced feed intake, increased urea and creatinine concentrations and increased prostate weights at 1000 ppm and greater (Wetzig & Geiss, 1998a).

Groups of four male and four female beagle dogs were given diets containing thiacloprid, (purity, 96.8–97.2 %) at a concentration of 0, 250, 1000 or 2000 ppm for 15 weeks. The animals at the highest dose were treated initially with a dose of 4000 ppm from day 1 to day 4, which caused severe clinical signs, like vomitus and body-weight reduction. Therefore, after a period without treatment from day 5 to day 14, this group was treated at 2000 ppm from day 15 onwards. This period was compensated for by a 2-week extension of the study period. The study was performed in compliance with test guideline OECD 409. The concentration, stability and homogeneity of the test material in the diet were acceptable. Daily intakes were equal to 0, 8.5, 34.9 and 68.0 mg/kg bw per day in males and 0, 8.9, 34.7 and 65.3 mg/kg bw per day in females, at 0, 250, 1000 and 2000 ppm, respectively.

Within the first days of treatment, animals at the highest dose (4000 ppm) displayed clinical signs, including vomitus, slight tremor, reduced or no feed intake, and body-weight decrease. Therefore, the treatment with the highest dose was stopped on day 4, and the dose was reduced to 2000 ppm. During the further course of the study, no clinical signs or effects on feed intake and body-weight development were observed. The lack of clinical signs at the chosen doses was not the consequence of poor absorption as was demonstrated by plasma concentrations determinations in study week 14. Blood samples were taken 0, 2, 4, 6 and 24 h after feeding. The peak values as measured 4–6 h after administration were approximately 2, 6, and 14 µg/ml for dogs at 250 ppm, 1000 ppm and 2000 ppm, respectively. Compared with the administered doses the blood concentrations are regarded as very high, thus, indicating a high absorption rate.

Haematological examination in study weeks 2, 7 and 15 did not show any treatment-related changes. Clinical chemistry revealed slight increases in transaminase activities at some time-points in dogs at 2000 ppm, which resolved during the study period. T4 concentrations were slightly decreased and thyroxin-binding capacity slightly increased at 1000 and 2000 ppm. Evidence of a slight liver enzyme induction (*N*-demethylase, EH) was observed at 2000 ppm while UDP-GT was marginally increased in females at 1000 ppm and in both sexes at 2000 ppm. The monooxygenase parameter EROD was significantly reduced in animals at the highest dose, which is often observed in studies in dogs, when a certain "stress effect" is observed in the liver. Urine analysis did not reveal any changes.

Liver weights (absolute and relative) were slightly increased in all dosed groups of males and in females at 1000 and 2000 ppm compared with the control groups; however, dose-dependency was not observed. Data for historical controls provided in the report for beagle dogs of this age gave absolute liver weights of 386.6 ± 51.8 g (range, 334.8–438.4 g) and relative liver weights of 36.7 ± 4.45 g/kg bw (range, 32.25–41.15 g/kg bw).

Histological examination revealed slight to moderate diffuse hypertrophy of the prostatic glandular epithelium in all males at doses of 1000 and 2000 ppm. In the affected animals the organ weights of the prostates (absolute and relative) were two to threefold higher when compared with controls and males at the lowest dose. In the testes of three of the four males at the highest dose, the Leydig cells appeared to be slightly more prominent. In some males at the highest dose, a slightly increased incidence of spermatocytic degeneration was found in the testes (two out of four dogs) and/or in the epididymides (four out of dogs compared with one dog in the control group). However, such findings are known to show a wide variation with respect to severity and incidence in young dogs. In the other examined organs and tissues no changes were detected.

Table 19. Main findings of a 15-week study in dogs fed diets containing thiacloprid

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	250	1000	2000	0	250	1000	2000
Body weight (kg)	9.55	9.50	9.43	9.13	9.15	9.03	9.13	9.55
Body-weight gain (kg)	1.71	1.52	1.95	1.50	1.77	1.43	1.78	1.95
T3 (nmol/l)	1.63	1.66	1.74	1.69	1.76	1.60	1.67	1.77
T4 (nmol/l)	19	21	16	10	24	20	16	15
TBC	0.80	0.87	0.89	0.91	0.81	0.89	0.90	0.91
N-DEM (mU/g)	44.4	58.2	50.2	69.6	62.6	46.1	54.6	79.1
O-DEM (mU/g)	20.0	18.8	20.0	24.0	22.1	26.4	23.7	30.2
P 450 (nmol/g)	15.2	16.9	15.9	14.9	18.1	17.7	19.6	19.8
ECOD (nmol/g per min)	17.8	17.4	17.8	18.1	19.3	17.8	15.6	21.5
EROD (nmol/g per min)	1.53	0.98	1.25	0.69	1.76	1.10	0.96	0.53
ALD (nmol/g per min)	18.0	20.1	16.8	19.0	15.5	15.3	15.1	20.1
EH (nmol/g per min)	1912	1971	2256	2624	2012	1658	2131	2623
GST (µmol/g per min)	80.6	83.3	95.8	79.2	77.8	87.5	88.2	106.3
UDP-GT (nmol/g per min)	2432	2311	2302	3010	2200	1993	2827	3496
Liver weight (g)	325.3	410.3	396.8	386.8	326.0	346.3	384.8	375.5
Relative liver weight (g/kg)	34.3	43.9	43.1	43.2	35.8	38.9	42.5	40.2
Testes / ovarian weight (g)	17.2	19.4	20.2	19.7	0.855	1.035	1.088	0.998
Relative testes / ovarian weight (g/kg)	1.82	2.06	2.18	2.22	0.095	0.117	0.121	0.106

Prostate / uterine weight (g)	2.98	2.88	7.40	8.35	3.8	5.0	4.8	6.5
Relative prostate / uterine weight (g/kg)	0.32	0.30	0.80	0.94	0.40	0.55	0.50	0.68
Prostate, hypertrophy	0	0	4	4	—	—	—	—

From Wetzig & Rinke (1998)

ALD, aldrin epoxidase; ECOD, 7-ethoxycoumarin deethylase; EH, epoxide hydrolase; EROD, 7-ethoxyresorufin deethylase; N-DEM, N-demethylase; O-DEM, O-demethylase; GST, glutathione-*S*-transferase; T3, triiodothyronine; T4, thyroxin; TBC, thyroxin-binding capacity; UDP-GT, uridine diphosphate glucuronosyl transferase.

The NOAEL was 250 ppm, equal to 8.5 and 8.9 mg/kg bw in males and females, respectively, on the basis of reduced T4 concentrations, liver effects (increased weight, enzyme induction) and prostate effects (increased weight, hypertrophy of the glandular epithelium) at 1000 ppm and greater (Wetzig & Rinke, 1998).

Groups of four male and four female beagle dogs were given diets containing thiacloprid (purity, 96.8–97.1%) at a concentration of 0, 40, 100, 250 or 1000 ppm for 52 weeks. In addition, for observation of possible prostatic effects, three groups of three male beagle dogs were fed diets containing thiacloprid at a concentration of 0, 100 or 1000 ppm for 26 weeks. The study was performed in compliance with test guideline OECD 452. The concentration, stability and homogeneity of the test material in the diet were acceptable. Daily intakes were equal to 0, 1.42, 3.60, 8.88 and 34.42 mg/kg bw per day in males and to 0, 1.39, 3.27, 8.30 and 33.80 mg/kg bw per day in females, at 0, 40, 100, 250 and 1000 ppm, respectively. For the male animals in the 26-week feeding study, the test compound intakes were 0, 3.23 and 32.40 mg/kg bw per day at 0, 100 and 1000 ppm.

The dietary dose schedule was based on the results of the short-term study and on the results of a study in which thiacloprid was given by capsule. In this study, thiacloprid was given at doses of 95 and 152 mg/kg bw for 4 weeks to determine whether capsule administration might overcome possible palatability problems that were anticipated to be the reason for the observed reduced intake of feed admixture in the feeding studies. The doses of 95 and 152 mg/kg bw were equal to 2500 ppm and to 4000 ppm in the diet, respectively. Determinations of the concentrations of the active ingredient in the plasma on days 1 and 12 revealed higher concentrations on day 12 (approximately 25–30 µg/ml) than after the first administration on day 1 (approximately 5–10 µg/ml). The animals displayed severe signs of toxicity, like vomitus, increased salivation, tremor and ptosis after both doses with a higher severity at the highest dose. In the group at the highest dose, one of the four animals died. Therefore, this study demonstrated that administration of higher doses by means of capsules is not possible and dietary administration was chosen for this study.

The animals were observed daily for clinical signs. Clinical chemistry and haematology measurements were done in weeks −4, 6, 13, 26, 39 and 52. Furthermore, reflex, body temperature, pulse rate and heart rate and electrocardiogram (ECG) determinations were performed. In addition, ultrasonography determinations every 9 weeks were also included for clarification of possible prostatic effects similar to those seen in the short-term study.

No clinical signs were observed, and all animals survived the study period. Furthermore, determinations of reflexes, body temperature, pulse rate and heart rate did not reveal substance-induced changes. Also the ECG measurements did not indicate treatment-related effects. No significant differences could be observed regarding the nutritional state of the animals. Feed intake was not impaired in male dogs in all dose groups, but females at 1000 ppm had slightly lower feed intake. Body-weight development was normal in all treatment groups when compared with the control group. Ophthalmoscopic investigations did not show treatment-related changes in any of the animals.

Haematological investigations did not reveal any remarkable differences between control and test animals.

The clinical chemical investigations revealed a slight decrease in T4 concentrations in the males at 1000 ppm in the 26-week, but not in the 52-week study (Table 20). Thyroxin-binding capacity and T3 concentrations were not altered. Thiacloprid did not cause an induction of microsomal liver enzymes after 52 weeks. Urine analysis did not reveal any changes between control and treated animals.

Table 20. Relevant findings in dogs fed diets containing thiacloprid in the main study (52 weeks) and the satellite study (26 weeks)

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	40	100	250	1000	0	40	100	250	1000
Main study (52-weeks)										
Body weight (kg), week 52	11.78	11.13	10.88	10.83	11.65	10.68	11.28	11.90	11.80	11.13
Body-weight gain (kg), weeks 1–52	3.00	2.33	2.00	1.98	2.25	2.38	2.83	3.55	3.27	2.40
T3 (nmol/l), week 26	1.92	1.93	1.83	1.79	1.79	1.86	1.95	1.91	1.77	1.72
T3 (nmol/l), week 52	1.61	1.55	1.58	1.57	1.72	1.47	1.55	1.65	1.47	1.71
T4 (nmol/l), week 26	23	21	21	19	15	23	23	22	20	20
T4 (nmol/l), week 52	15	17	17	20	14	18	21	22	21	20
Liver weight (g)	456.0	437.0	435.0	449.8	433.3	402.0	414.3	406.8	456.5	388.3
Relative liver weight (g/kg)	39.28	38.90	40.63	42.13	38.05	37.35	36.93	34.25	39.18	35.88
Prostate weight (g)	9.25	12.18	8.65	11.40	16.13	—	—	—	—	—
Relative prostate weight (g/kg)	0.795	1.063	0.808	1.048	1.333	—	—	—	—	—
Kidney/pigment, grade 1	2	3	2	3	1	2	2	2	2	0
Grade 2	2	1	2	0	1	2	2	2	1	0
Grade 3	0	0	0	1	2	0	0	0	1	4
Average grade	1.5	1.3	1.5	1.5	2.3	1.5	1.5	1.5	1.8	3.0
Satellite study (26-weeks)										
T3 (nmol/l), week 26	1.77	—	1.71	—	1.82	—	—	—	—	—
T4 (nmol/l), week 26	23	—	19	—	16	—	—	—	—	—
Liver weight (g)	389.3	—	387.7	—	405.7	—	—	—	—	—
Relative liver weight (g/kg)	35.63	—	35.57	—	37.70	—	—	—	—	—
Prostate weight (g)	9.23	—	8.73	—	10.27	—	—	—	—	—
Relative prostate weight (g/kg)	0.800	—	0.803	—	0.957	—	—	—	—	—

From Wetzig & Geiss (1998)

Historical controls for absolute and relative prostate weights:

26 weeks (137 dogs): absolute weight (g) = 4.1 ± 2.15; relative weight (g/kg) = 0.380 ± 0.190

52 weeks (108 dogs): absolute weight (g) = 8.6 ± 3.44; relative weight (g/kg) = 0.735 ± 0.290

An increase in liver weights was observed in male dogs at the highest dose after the 26-week treatment period but not after 52 weeks. Histopathology revealed hepatocellular cytoplasmic change in all male dogs after 26 weeks but not after 52 weeks, indicating an adaptive response to xenobiotic exposure that is later compensated for. In the kidneys, the severity of pigment (lipofuscin) deposition in the proximal tubules was increased after 52 weeks at 1000 ppm. At interim necropsy after 26 weeks, prostatic weights and sizes in the groups at 100 and 1000 ppm were not different from those of the controls. After 52 weeks of treatment, mean prostatic weights (absolute and relative) and sizes in the group of male animals at the highest dose were slightly increased compared with control values and with

the treatment groups receiving thiacloprid at 40 to 250 ppm. Additional morphological investigations by sonography of the dog prostates over the entire study period did not indicate compound-induced differences between control and treated groups. Also, histopathological examination of the prostate gland did not reveal treatment-related morphological changes after 26 and 52 weeks in male dogs. The report stated that the average prostate weight was increased in the group at the highest dose, mainly due to the extremely high prostate weight of one animal (B813) and that the average prostate weights of the control group (9.25 g) was in the upper range of historical control data (8.6 ± 3.44 g) so that, besides treatment with 1000 ppm, individual differences in the maturation of the animals in this study may also have played a role. However, three of the dogs at 1000 ppm, one of the dogs at 250 ppm and two of the dogs at 40 ppm had prostate weights that were clearly above the range for cited historical control data. The individual prostate weights and body weights are presented in Table 21.

Table 21. Individual body and prostate weights at terminal sacrifice of dogs fed diets containing thiacloprid for 52 weeks

Dietary concentration (ppm)	Dog No.	Body weight (kg)	Prostate weight (g)	Relative prostate weight (g/kg)	Prostate height (mm)	Prostate width (mm)
0	B779	12.6	9.4	0.75	21	26
	B773	11.5	9.6	0.83	21	29
	B769	12.6	8.3	0.66	20	26
	B799	10.3	9.7	0.94	23	27
40	B753	10.9	4.7	0.43	20	20
	B797	8.9	10.6	1.19	22	30
	B807	13.0	17.0	1.31	27	32
	B759	12.4	16.4	1.32	30	33
100	B775	10.0	5.3	0.53	17	22
	B805	10.9	11.1	1.02	24	29
	B781	9.0	8.9	0.99	23	27
	B783	13.4	9.3	0.69	22	27
250	B815	11.2	9.1	0.81	23	27
	B767	11.7	19.3	1.65	27	35
	B761	10.0	9.1	0.91	24	28
	B795	9.9	8.1	0.82	21	26
1000	B771	6.8	6.3	0.93	20	26
	B813	13.9	25.1	1.81	27	36
	B749	11.9	17.2	1.45	30	32
	B787	14.0	15.9	1.14	27	32

From Wetzig & Geiss (1998)

The NOAEL was 250 ppm, equal to 8.9 and 8.3 mg/kg bw per day in males and females, respectively, on the basis of decreased concentrations of T4, liver effects (hepatocellular cytoplasmic changes) and prostate effects (increased weight and size) at 1000 ppm (Wetzig & Geiss, 1998b).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity, groups of 50 male and 50 female B6C3F_1 mice were fed diets containing thiacloprid (purity, 96.8–97.2%) at a concentration of 0, 30, 1250 or 2500 ppm for up to

107 weeks. In addition, groups of 10 male and 10 female mice were given thiacloprid at 0 or 2500 ppm and were sacrificed after 1 year. The study was performed in compliance with test guideline OECD 451. Clinical observations, body weight and feed intakes were recorded at appropriate time-points. Laboratory investigations (haematology, biochemistry and urine analysis) were performed at 53, 79 and 104/106 weeks. All animals received a macroscopic examination at necropsy. The following organs were removed and weighed: brain, heart, liver, spleen, kidneys, adrenal glands, ovaries and testes. An extensive list of organs and tissue were subjected to microscopic examination. The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes at 0, 30, 1250 and 2500 ppm were equal to 0, 5.7, 234.1 and 546.4 mg/kg bw per day in males and 0, 10.9, 475.3 and 872.5 mg/kg bw per day in females, respectively.

There was no evidence of a treatment-related increase in mortality rate. No treatment-related clinical signs were noted during the study. In males at 2500 ppm, mean body weight was significantly reduced (up to 9%) and mean feed intake was significantly increased (10%), indicative of reduced feed efficiency.

Small but significant increases in the mean leukocyte count were seen in males at 1250 and 2500 ppm at all sampling points and in females at weeks 53 and 79.

At interim necropsy, liver weights were increased in both sexes (absolute, 25% in males, 33% in females; relative, 32% in males, 32% in females), while adrenal weights were markedly increased in females (absolute 80%, relative 70%) at 2500 ppm. The microscopic examination revealed a centrilobular minimal to moderate hepatocellular hypertrophy associated with an increase of hepatocellular fat droplets in males while the livers of females were unaffected. In the adrenal glands of all treated females, a persistence of the X zone characterized by severe to marked vacuolization of the X zone was observed. In controls, the X zone had almost completely disappeared and only a small rim or single cells surrounding the medulla were found. In the kidneys of treated males a slight to severe reduction of sex-specific vacuoles in renal proximal tubules was found. The finding is known as a strain-specific effect in $B6C3F_1$ mice and is considered to be an unspecific and not an adverse effect (Table 22). Neoplastic findings at interim necropsy were seen in the Harderian gland (adenoma) in a female in the control group and in the skin (sarcoma) of a female at 2500 ppm.

Table 22. Relevant findings at interim sacrifice (52 weeks) in a study of carcinogenicity in mice fed diets containing thiacloprid

Finding	Dietary concentration (ppm)			
	Males		Females	
	0	2500	0	2500
Mortality (No. of animals)	0	0	1	0
Body weight (g)	37	35	29	29
Liver weight (mg)	1758	2194**	1366	1811*
Relative liver weight (mg/100 g)	4734	6249**	4791	6331**
Adrenals weight (mg)	9	9	10	18**
Relative adrenals weight (mg/100 g)	25	25	37	63**
Testes /ovaries weight (mg)	205	211	41	24
Relative testes /ovaries weight (mg/100 g)	553	606	147	86
Liver, hepatocellular hypertrophy	0	10	0	0
Vacuolization	1	9	0	0
Fatty change	5	10	3	3
Kidney, decrease in sex-specific vacuoles	0	8	0	0
Adrenals, vacuolization of X-zone	0	0	2	10

From Wirnitzer & Geiss (1998)

* $p < 0.05$; ** $p < 0.01$

No gross findings were observed in animals that died or were killed in a moribund condition during the study. At termination, gross necropsy revealed an increased incidence of nodules in the ovaries (1, 4, 4, and 6 at 0, 30, 1250 and 2500 ppm, respectively). Microscopic examination revealed that 9/15 of these nodules were caused by purulent abscesses/inflammation and 6/15 nodules were diagnosed as neoplastic lesions.

The histopathological investigations revealed hepatocellular hypertrophy in nearly all males and a few females in the groups at the intermediate and highest dose. Additionally, an increase of fatty change and of centrilobular hepatocellular degeneration was found in males at 1250 ppm and greater. The incidence and severity of hepatocellular necrosis was increased in both sexes at 2500 ppm. The observed liver effects were considered to be a consequence of chronic enzyme induction (as seen in mice in the 14-week study at dietary concentrations of 250 ppm and greater).

In the adrenal glands of females at 1250 ppm and greater, there was a significant increase in the incidence and severity of focal or diffuse vacuolization of the X-zone when compared with the controls. This finding was considered to be due to delayed regression of the X-zone. The report considered these changes to be secondary to the liver effects producing increased synthesis of steroid hormones or altered kinetics.

In the medullary regions of the mandibular and mesenteric lymph nodes, a slight increase in the incidence and severity of vacuolated cells was seen at 1250 ppm and greater; however, the toxicological relevance of this finding was unclear.

In the ovarian stroma or the surrounding adipose tissue, an increased number of large, eosinophilic, and luteinized cells were seen in females at 1250 and 2500 ppm. Also, an increased incidence of ovarian luteomas was evident at 1250 ppm and greater when compared with the control value (Table 23). The single tumour at 30 ppm is within the range for historical controls for this strain of mice. These effects were considered to be a consequence of the known liver enzyme induction by thiacloprid in rats and mice, resulting in a secondary hormone imbalance.

The total incidence of tumours, the time of occurrence and the type of tumours observed in this study did not indicate any significant differences between treated and control mice. The same was true for the number of animals with primary neoplasms, the number of animals with more than one primary neoplasm, the number of animals with metastases and the number of benign and malignant neoplasms per dose group and sex (Table 23).

Table 23. Relevant findings at terminal sacrifice in a study of carcinogenicity in mice fed diets containing thiacloprid

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	30	1250	2500	0	30	1250	2500
Mortality (No. of animals)	3 (2)[a]	11 (2)	3 (4)	4 (3)	15 (0)	19 (2)	17 (2)	17 (3)
Body weight (g)	37	37	37	34*	29	29	29	30*
Liver weight (mg)	2008	1924	2119	2239	1626	1732	1800	2096**
Relative liverweight (mg/100 g)	5551	5218	6017**	6477**	5638	6055	6136*	6906**
Adrenals weight (mg)	10	10	10	9	12	11	13	13
Relative adrenals weight (mg/100 g)	28	28	27	28	43	38	45	44
Testes /ovaries weight (mg)	202	198	208	202	222	300	148	117
Relative testes /ovaries weight (mg/100 g)	558	539	571	599*	792	991	513	385
Liver, No. examined	50	50	50	50	49	48	50	50
Hepatocellular hypertrophy	0	0	46**	49**	0	0	2	3*
Fatty change	3	4	15**	21**	2	3	3	7*

Degeneration	1	0	5*	16**	0	0	0	0
...Necrosis	5	3	6	31**	15	17	17	25*
Mesenteric lymph node, No. examined	50	49	49	50	49	44	46	48
Vacuolization	31	33	47**	43**	18	17	34**	35**
Mandibular lymph node, No. examined	43	50	49	46	48	45	49	45
Vacuolization	28	36	45**	35	19	20	27	31**
Adrenals, No. examined	49	50	50	50	49	48	50	50
X-zone vacuolization	0	0	0	0	33	36	48**	50**
Grade 1	—	—	—	—	31	34	18	9
Grade 2	—	—	—	—	1	1	19	31
Grade 3	—	—	—	—	—	1	5	8
Grade 4	—	—	—	—	1	—	6	2
Mean grade	—	—	—	—	1.1	1.1	2.0	2.1
X-zone atrophy	—	—	—	—	48	48	44	46
Grade 3	—	—	—	—	0	1	5	6
Grade 4	—	—	—	—	1	1	11	15
Grade 5	—	—	—	—	47	46	28	25
Mean grade	—	—	—	—	5.0	4.9	4.5	4.4
Ovaries, No. examined	—	—	—	—	47	48	49	47
Eosinophilic luteinized cells	—	—	—	—	3	0	5	8
Luteoma (benign)	—	—	—	—	0	1	5*	5*
Luteoma (malign)	—	—	—	—	0	0	0	1
Cystadenoma (benign)	—	—	—	—	4	1	1	4
No. of animals at necropsy	50	50	50	50	50	50	50	50
No. of benign tumours [b]	20	18	16	14	22	17	26	22
No. of malignant tumours [b]	10	21	13	8	34	26	23	31
Total tumours [b]	30	39	29	22	56	43	49	53

From Wirnitzer & Geiss (1998)

* $p < 0.05$; ** $p < 0.01$.

[a] Additional animals that died due to blood sampling.

[b] Tumours in paired organs are counted as one primary tumour.

The NOAEL for systemic toxicity was 30 ppm, equal to 5.7 and 10.9 mg/kg bw per day in males and females, respectively, on the basis of effects in liver (increased weight, hepatocellular hypertrophy, fatty change, degeneration) and lymph nodes (vacuolization) in both sexes and in the adrenal cortex (vacuolization in the X-zone) in females at 1250 ppm and greater. There was no evidence of oncogenic activity in males. In females, the incidence of benign ovarian luteomas was significantly increased at 1250 ppm and greater (Wirnitzer & Geiss, 1998).

Rats

In a long-term combined study of toxicity and carcinogenicity, groups of 50 male and 50 female Wistar (Hsd Cpb:WU) rats were fed diets containing thiacloprid (purity, 96.8–97.2%) at a concentration of 0, 25, 50, 500 or 1000 ppm for up to 107 weeks. In addition, groups of 10 male and 10 female rats were treated likewise and sacrificed after 1 year. The study was performed in compliance with test guideline OECD 453. Clinical observations, body weight, feed and water intakes and laboratory investigations were recorded at suitable time-points. Ophthalmological investigations were performed before the start of the study, at 1 year and at the end of treatment. All animals

received a macroscopic examination at necropsy. The following organs were removed and weighed: brain, heart, liver, spleen, kidneys, adrenal glands, ovaries and testes. An extensive list of organs and tissue were subjected to microscopic examination. Liver samples were taken from the interim animals for enzyme determinations (10 rats of each sex at 0 and 25 ppm and five rats of each sex at 50, 500 and 1000 ppm). The concentration, stability and homogeneity of the test material in the diet were acceptable. Mean daily intakes at 0, 25, 50, 500 and 1000 ppm were equal to 0, 1.2, 2.5, 25.2 and 51.7 mg/kg bw per day in males and 0, 1.6, 3.3, 33.5 and 69.1 mg/kg bw per day in females, respectively.

No treatment-related clinical signs were detected by daily observation of the animals, and mortality was not increased (Table 24). The rather low survival rate of females in the control group compared with all treated groups was considered to be incidental but could possibly have had an influence on certain age-related lesions. In males, the most frequent cause of death of animals that died or were killed before the scheduled necropsy was degenerative changes in the kidneys or heart. In females, the main causes of death were tumours in the pituitary and uterus and to a lesser extent in the mammary gland. The increased incidence of uterine tumours at 500 ppm and greater was the main cause of death in these groups, but did not cause an overall increase in mortality. Pituitary adenomas as death cause were markedly reduced after 500 ppm and greater.

Thiacloprid had no effect on feed consumption at concentrations up to and including 50 ppm, while at the highest concentration the feed consumption was slightly lower than in the controls. There was no relevant effect on water intake. The body weights and the weight gains were not affected at doses of up to and including 50 ppm. At 500 ppm a slight and transient growth retardation was observed in males, while in females there was a pronounced growth retardation starting with week 8 and reaching body-weight differences from controls of up to −15% between weeks 57 and 77. Body-weight differences from controls at 1000 ppm were up to −12% in males and −21% in females (Table 24).

Ophthalmological investigations gave no indication of an oculotoxic effect of the test compound in any of the groups of males, while in females some ophthalmological findings were more frequent in all treated groups compared with controls, e.g. lenticular alterations in the cortex/nucleus and opacities in the retrolenticular area. Since the incidences in controls were rather low (probably as a result of the low survival), a dose–response relationship was partly lacking and there was no increase in the severity of changes, a treatment-related effect was not considered to be likely at doses of up to 500 ppm. Thus, only the increased incidence of lens alterations in females at 1000 ppm was considered to be a treatment-related effect.

Haematological investigations provided no evidence of damage to the blood, coagulation or the blood-forming tissues. Also, urine analysis did not reveal any compound-related effects.

The determination of plasma enzymes revealed a tendency to lower activities, especially of aspartate aminotransferase (males and females) and alkaline phosphatase (males), essentially at the highest dose, which was regarded as a possible secondary consequence of the liver enzyme induction. This conclusion was corroborated by some slight changes in other parameters of liver function, such as slightly increased cholesterol values in males and females at 1000 ppm at week 26, slightly decreased triglyceride concentrations essentially in females at 500 and 1000 ppm (all time-points), slightly decreased total bilirubin concentrations, essentially in males at 500 and 1000 ppm (all time-points) and increased total protein concentrations, essentially in males and females at 1000 ppm (all time-points).

Table 24. Relevant clinical and clinical-chemistry findings in a long-term study in rats fed diets containing thiacloprid

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	25	50	500	1000	0	25	50	500	1000
Cumulative mortality:										
Weeks 1–13	0	0	0	0	0	0	0	0	0	0
Weeks 14–27	0	0	0	0	0	0	0	0	1	0
Weeks 28–41	0	1	0	1	0	2	0	1	1	0
Weeks 42–55	2	1	0	1	1	3	0	2	2	1
Weeks 56–69	2	2	0	1	1	7	2	4	4	2
Weeks 70–83	2	3	2	2	3	15	7	9	8	5
Weeks 84–97	8	11	8	8	5	26	15	12	13	16
Weeks 98–104	11	12	14	10	9	28	22	14	19	17
Feed consumption (g/rat per day)	20.0	19.6	20.3	19.4	19.4	15.5	15.4	15.2	14.3	14.3
Body weight (g):										
Interim sacrifice	525	528	514	538	503	273	321*	293	267	247
Terminal sacrifice	507	528	523	493	461**	311	331	307	282*	265**
Eyes, No. examined at term	78	74	76	80	86	46	55	72	65	66
Lens alterations; No. (%)	13(17)	23(31)	28(37)	18(23)	20(23)	1(2)	8(15)	9(13)	12(18)	21(32)
Retrolenticular opacities, No. (%)	20(26)	20(27)	15(20)	20(25)	22(26)	1(2)	0(0)	9(13)	7(11)	9(14)
T3 (nmol/l):										
Week 26	1.30	1.42	1.28	1.39	1.39	1.40	1.30	1.36	1.37	1.41
Week 105	2.16	2.09	2.17	2.12	2.19	1.91	1.86	1.98	2.04	2.08
T4 (nmol/l):										
Week 26	54	55	51	53	56	43	38	38	32*	38
Week 105	49	48	50	43	47	38	41	42	41	40
TSH (µg/l):										
Week 26	2.49	3.47	2.78	5.31	6.13	1.31	1.24	2.29*	1.69	2.96**
Week 105	1.78	1.55	1.51	1.99	2.81	0.62	0.49	1.02	0.84	1.46**
ECOD (nmol/g per min)[a]	4.4 [4.4]	6.1** [3.6]	6.5**	11.3**	18.2**	3.4 [2.9]	3.6 [3.1]	4.1	7.0**	11.6**
EROD (nmol/g per min)[a]	0.50 [0.45]	0.45 [0.15**]	0.67	0.70	0.72	0.26 [0.25]	0.34 [0.18]	0.24	0.24	0.39
ALD (nmol/g per min)[a]	122 [139]	167** [99]	194**	167*	243**	25.5 [29.5]	34.3 [28.3]	30.7	53.4**	63.6**
EH (nmol/g per min)[a]	674 [525]	676 [436]	688	783	1325**	183 [243]	285* [301]	303*	532**	613**
GST (µmol/g per min)[a]	108 [98]	120 [82]	102	144**	161**	83 [84]	80 [86]	91	111**	130**
UDP-GT (nmol/g per min)[a]	336 [522]	388 [388]	362	547**	635**	170 [370]	186 [549]	199	324**	433**

From Bomhard et al. (1998)

* $p < 0.05$; ** $p < 0.01$;

ECOD: 7-ethoxycoumarin deethylase; EROD: 7-ethoxyresorufin deethylase; ALD: aldrin epoxidase; EH: epoxide hydrolase; GST: glutathion-S-transferase; UDP-GT: uridine diphosphate glucuronosyl transferase.

[a] Values in square brackets indicate additional investigations.

Although decreased concentrations of T3 and T4 were expected due to induction of liver enzymes, no such decreases were observed in this study. The concentration of TSH was non-significantly and mostly slightly increased in males at 1000 ppm at all time-points. After 26 weeks the effect was most pronounced with a few rather high individual values. At that time TSH was also increased in some males at 500 ppm. In females at 1000 ppm, TSH was significantly increased at weeks 26 and 105.

The determination of enzymes in liver homogenates revealed an induction of all measured enzymes (except EROD) in males and females. In male, ALD and ECOD were significantly and dose-dependently induced up to a factor of 2 (ALD) or 4 (ECOD) at 1000 ppm. EH was significantly induced only at 1000 ppm by a factor of 2. The phase II enzymes GST and UDP-GT were significantly induced at 500 and 1000 ppm (up to factors of 1.5 and 1.9, respectively). A similar induction pattern occurred in females: ECOD and ALD were significantly induced at 500 and 1000 ppm up to a factor of 3.4 and 2.5, respectively. Induction of EH was more pronounced in female rats than in males. The activity increased up to a factor of 3.3 at 1000 ppm and was statistically significantly higher at 25 ppm and greater. The phase II enzymes were significantly induced at 500 and 1000 ppm. The UDP-GT activity at 1000 ppm was increased by a factor of 2.5 and that of GST by a factor of 1.6. Since there were also slightly but significantly increased enzyme activities (ECOD, ALD in males, EH in females) at 25 ppm, an additional five liver samples were investigated in rats in the control groups and at 25 ppm to find out whether these findings indicated a real effect or were incidental. No significant enzyme induction was detected in the stored liver samples from rats at 25 ppm (Table 24).

At the interim sacrifice after 1 year of treatment, gross necropsy did not reveal any treatment-related abnormalities. The relative liver weights were significantly increased in females at 1000 ppm (16%) and non-significantly in males (9%). The microscopic investigations after 1 year of treatment revealed hepatocellular hypertrophy in both sexes at 500 ppm and greater. This finding was accompanied by focal fat infiltration in males. Also, hypertrophy of thyroid epithelium associated with an increased incidence of colloid clumping was observed in males at 500 ppm and greater and in females at 1000 ppm. Although there was no clear evidence of carcinogenic activity in rats at interim, follicular cell adenoma was found in one male thyroid at 1000 ppm and hepatocellular adenoma was found in one female at 1000 ppm (Table 25).

At the terminal sacrifice, gross necropsy showed an increased incidence of "area/s" in the livers of males at 1000 ppm and of "cyst/s" in females at 500 ppm and greater. In the thyroid, an increased incidence of "enlargement" and "nodules" was observed in males at 500 ppm and greater. The uterus showed an increased incidence of "nodules" at 1000 ppm. In the eyes, the incidence of "turbidity" was increased in females at 500 ppm and greater. There was a decrease in the incidence of "nodules" at the skin in females at 1000 ppm and in the incidence of "nodules" in the pituitary gland in females at 500 ppm and greater.

Absolute and relative liver weights were significantly higher at 1000 ppm in males (relative weight, 31%), as were relative weights in females (16%).

The histopathological investigations of the animals at termination (including animals that died intercurrently) revealed a hepatocellular cytoplasmic change (eosinophilic cytoplasm with basophilic strands) and a predominantly centrilobular hepatocellular hypertrophy in males at 50 ppm and greater and in females at 500 ppm and greater. The incidence of hepatocellular vacuolation was increased in males at 1000 ppm while that of focal necrosis and biliary cysts was slightly elevated in females at 500 ppm and greater. Mixed eosinophilic clear-cell foci were significantly increased in males at 50 ppm and greater and in females at 1000 ppm. The incidence of basophilic foci was reduced in both sexes and the incidence of clear-cell foci was reduced in females at 500 ppm and greater (Table 25).

The histopathological investigations of the nervous system and skeletal musculature revealed evidence of substance-related damage in males and females at 500 ppm and greater. The following findings were made: an increased incidence of cholesterol clefts in the nerve roots of the spinal cord (females at 1000 ppm), of radiculoneuropathy (females at 500 and 1000 ppm), of sciatic nerve degeneration (both sexes at 500 and 1000 ppm), skeletal muscle atrophy (in females at 500 and 1000 ppm) and degeneration as well as mononuclear infiltrates in the sciatic nerve (in females at 1000 ppm). All these findings are known to occur spontaneously in old rats and are termed spinal radiculoneuropathy or degenerative myelopathy and may be exacerbated by xenobiotics (Greaves, 1990). An increased incidence of cholesterol clefts was seen in the space between the anterior pituitary and the pars intermedia in males at 500 or 1000 ppm. In the mesenteric lymph nodes, there was a significant increase in the incidence of sinus histiocytosis in females at 1000 ppm (Table 25).

Table 25. Relevant non-neoplastic findings in a long-term study in rats fed diets containing thiacloprid

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	25	50	500	1000	0	25	50	500	1000
Interim sacrifice										
Liver weight (g), week 52	18.5	17.4	17.1	19.4	19.3	9.0	10.1	9.7	9.5	9.5
Relative liver weight (g/100g), week 52	3.5	3.3	3.3	3.6	3.8	3.3	3.2	3.3	3.5	3.8**
Liver; No. examined	10	10	10	10	10	10	10	10	10	10
Hepatocellular hypertrophy	0	0	0	5	9	0	0	0	5	8
Focal fat infiltration	0	0	0	5	7	0	1	2	0	0
Thyroid; No. examined	10	10	10	10	10	10	10	10	10	10
Hypertrophy	1	1	1	7	8	0	0	0	0	4
Colloid clumping	3	5	3	10	10	1	1	1	1	8
Terminal sacrifice										
Liver weight (g)	16.9	17.1	16.7	17.4	20.2**	12.0	11.5	10.9	11.0	12.0
Relative. weight (g/100g)	3.4	3.3	3.2	3.6	4.4**	3.9	3.5	3.6	3.9	4.5**
Liver; No. examined	50	50	50	50	50	50	50	50	50	50
Biliary cyst/s	3	3	0	5	3	6	3	3	10	9
Cytoplasmic change	0	0	8**	41**	47**	0	1	0	30**	34**
Centrilobular hypertrophy	0	0	12**	44**	49**	0	1	0	30**	36**
Mixed eosinophilic clear-cell foci	1	2	5*	15**	22**	2	1	3	6	10**
Basophilic foci	16	9	9	6	7	19	19	19	6	2
Clear cell foci	33	34	33	36	33	15	14	15	7	4
Necrosis	7	4	1	2	2	9	3	6	12	15
Vacuolation	8	14	8	12	24**	6	7	4	3	4
Thyroid; No. examined	50	50	50	50	49	50	50	50	50	48
Follicular cell hyperplasia	1	2	2	6	3	0	0	1	1	3*
Follicular epithelium hypertrophy	12	10	22*	27**	34**	6	2	6	16**	23**
Colloid alteration	17	16	21	37**	41**	6	2	5	17**	28**
Pigment	16	11	23	30**	32**	0	0	2	4*	4*
Spinal cord; No. examined	50	50	50	50	50	50	50	50	50	50
Radiculoneuropathy	33	35	43	39	33	31	32	32	37	39*
Cholesterol clefts	9	11	8	14	11	3	4	8	4	13**

Sciatic nerve; No. examined	50	50	49	48	49	48	50	50	49	50.0
Degeneration	24	28	26	37**	34*	20	18	19	28	36**
Eyes; No. examined	50	50	50	50	50	50	50	50	50	50
Retinal atrophy	17	21	19	19	25	15	20	24*	25*	32**
Lens degeneration	21	27	22	21	22	9	18	16	20**	30**
Pituitary gland	50	50	50	50	50	50	50	50	50	50
Cholesterol clefts	0	1	2	4*	4*	0	0	0	0	0
Ovaries; No. examined						50	49	50	48	50
Cyst/s						16	15	19	22	24
Mammary gland; No. examined	50	50	50	50	50	50	50	50	50	50
Lacteal cyst/s	1	3	4	1	1	18	15	10	12	6
Galactocele	0	0	0	0	0	3	3	4	2	0
Mesenteric lymph node; No. examined	50	50	48	49	50	49	50	49	50	48
Sinus histiocytosis	8	5	9	7	13	12	10	14	19	25**
Skeletal muscle; No. examined	50	50	50	50	50	50	50	50	50	50
Atrophy	19	19	16	20	25	6	3	5	14*	20**
Degeneration	9	14	16	14	14	9	5	10	10	18*
Mononuclear infiltration	5	5	7	1	7	0	1	2	2	4*

From Bomhard et al., (1998)
* $p < 0.05$; ** $p < 0.01$.

Retinal degeneration and degeneration of lens fibres were seen in the eyes of controls and dosed animals. These age-related changes were significantly increased in females at 50 ppm (retinal atrophy) or 500 ppm (degeneration of lens fibres) and greater. However, since the incidences for these findings at 50 and 500 ppm were within the range for historical controls from studies conducted at the same laboratory (range for retinal atrophy: males, 3–21 out of 50 animals; females, 12–29 out of 50 animals; range for degeneration of lens fibres: males, 5–38 out of 50 animals; females, 6–23 out of 50 animals), only the increased incidences at 1000 ppm in females were considered to be treatment-related.

In the thyroid, the incidence of hypertrophy of the follicular epithelium was significantly increased in males at 50 ppm and greater and in females at 500 ppm and greater. Colloid alteration (clumping, inhomogenous colloid) and pigment in the follicular epithelium were significantly increased in both sexes at 500 ppm and greater. In males, there was an increase of follicular cell hyperplasias (Table 25) and a significant increase in thyroid follicular cell adenomas at 500 and 1000 ppm (Table 26), while in females, hyperplasias were significantly increased at 1000 ppm. These effects on the thyroid were considered to be secondary effects of enzyme induction on the liver, especially of UDP-GT, which leads to increased degradation and biliary excretion of circulating thyroid hormones. The resulting thyroid hyperactivity was documented by the increased concentrations of TSH in males at 500 ppm and greater and in females at 1000 ppm.

Table 26. Neoplastic findings in a long-term study in rats fed diets containing thiacloprid

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	25	50	500	1000	0	25	50	500	1000
Thyroid; No. examined	50	50	50	50	49	50	50	50	50	48
C-cell adenoma (b)	10	4	—	8	9	7	6	7	5	3
Follicular cell adenoma (b)	—	—	1	5*	8**	—	1	1	1	2
C-cell carcinoma (m)	—	—	—	1	—	—	—	—	—	—

Uterus; No. examined	—	—	—	—	—	50	50	50	50	50
Stromal polyp (b)	—	—	—	—	—	11	11	11	7	9
Glandular polyp (b)	—	—	—	—	—	—	1	—	—	1
Adenoma (b)	—	—	—	—	—	—	—	1	1	2
Adenocarcinoma (m)	—	—	—	—	—	6	3	3	14	18
Squamous cell carcinoma (m)	—	—	—	—	—	2	—	—	1	—
Mixed Muellerian tumour (m)	—	—	—	—	—	—	—	—	1	1
Adenosquamous carcinoma (m)	—	—	—	—	—	—	—	—	1	2
Stromal sarcoma (m)	—	—	—	—	—	1	1	1	—	—
Schwannoma (m)	—	—	—	—	—	—	—	2	—	—
Granular cell tumour (b)	—	—	—	—	—	—	—	—	—	1
No. of animals with neoplasms	32	30	31	33	37	42	37	41	41	42
No. of animals with more than one primary neoplasm	14	9	8	11	19	20	17	13	22	18
No. of animals with metastases	2	1	1	1	2	6	1	4	11	13
No. of primary neoplasms	56	42	42	47	61	70	63	62	72	73
No. of benign neoplasms	44	35	37	37	52	53	54	51	50	43
No. of malignant neoplasms	12	7	5	10	9	17	9	11	22	30
Occurence of tumours over time:										
Weeks 0–52	1	—	—	—	—	2	—	—	—	—
Weeks 53–65	—	1	—	—	—	1	—	2	—	—
Weeks 6–78	—	—	—	—	2	6	4	6	2	4
Weeks 79–91	3	4	3	4	—	14	11	5	9	4
Weeks 92–104	5	4	10	1	2	13	14	7	14	12
Week 105–sacrifice	—	—	2	2	3	1	7	7	3	2

From Bomhard et al., (1998)

b, benign; m, malignant.

* $p < 0.05$; ** $p < 0.01$.

There was further evidence of secondary effects on the endocrine system in female rats. In the ovaries, the incidence of cysts was increased at 500 ppm and greater. In the mammary glands, there was a reduced incidence of lacteal cysts and galactocele at 1000 ppm. In the uterus, an increased number of adenocarcinomas was observed after 500 and 1000 ppm that was significant in the trend test but not in the pairwise comparison. The incidences were 12, 6, 6, 28 and 36% at 0, 25, 50, 500 and 1000 ppm, respectively. The incidences at 500 and 1000 ppm were above the range for historical control data from the RITA database (in all studies in Wistar rats, 0–14.3%) and also above the control range from seven studies with the same rat strain in the same laboratory (0–10%). The effects on the uterus and the mammary glands were explained by alterations in hormone metabolism resulting from the induction of the microsomal liver enzymes, including aromatase, an enzyme involved in estradiol synthesis. This results in increased plasma estradiol levels and continuous stimulation of the uterine endometrium, which may explain the increased incidence of uterine adenocarcinomas in old and acyclic rats.

In males, there was a small increase in the number of animals with neoplasms, those with more than one neoplasm, and the number of benign neoplasms, which can be explained by the increased incidence of thyroid follicular cell adenoma. The single follicullar cell adenoma seen at 50 ppm was considered to be within the range for historical controls for this strain of rat. In females, there was an increased incidence in the number of animals with metastasizing malignant neoplasms, which

are caused by the higher incidence of uterine adenocarcinoma. The combined incidences of uterine adenoma and adenocarcinoma at 25 and 50 ppm were lower than the incidence in controls.

The NOAEL for systemic toxicity was 25 ppm, equal to 1.2 and 1.6 mg/kg bw per day in males and females, respectively, on the basis of liver toxicity (increased mixed eosinophilic-clear cell foci) and thyroid effects (follicular epithelial hypertrophy) at 50 ppm and greater. The NOAEL for oncogenicity was 50 ppm, equal to 2.5 and 3.3 mg/kg bw per day in males and females, respectively, on the basis of increased incidences of thyroid follicular cell adenoma in males and uterine adenocarcinoma in females at 500 ppm and greater (Bomhard et al., 1998).

2.4 Genotoxicity

In vitro

The mutagenic potential of thiacloprid (purity, 97.2%; dissolved in dimethylsulfoxide [DMSO]) was investigated in a test for reverse mutation in *Salmonella typhimurium* (TA1535, TA100, TA1537 and TA98). The study complied with test guideline OECD 471. Six concentrations of up to 5000 µg/plate were used in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). A slight bacteriotoxic effect was noted at the highest concentration. Tests were performed with quadruplicate plating at each concentration using the plate incorporation method, and results were confirmed in an independently repeated assay using the preincubation method. No biologically or statistically significant increase in the number of revertant colonies was seen in any strain at any concentration. The positive controls (sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene) produced significant increases in the frequency of revertant colonies (Herbold, 1995a).

The mutagenic potential of thiacloprid (purity, 96.8%; dissolved in DMSO) was investigated in a test for reverse mutation in *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* (WP2 *uvr*A). The study complied with test guideline OECD 471. Five concentrations of up to 5000 µg/plate were used in the presence and absence of a metabolic activation system (phenobarbital- and 5,6-benzoflavone-induced rat liver S9). Precipitation of the test material was seen at the highest concentration used in this study. Tests were performed with triplicate plating at each concentration using the preincubation method and results were confirmed in an independently repeated assay. No biologically or statistically significant increase in the number of revertant colonies was seen at any concentration or in any strain. The positive controls (furylfuramide, sodium azide, 9-aminoacridine and 2-aminoanthracene) produced significant increases in the number of revertant colonies (Ohta, 1995).

The mutagenic potential of thiacloprid (purity, 96.8–97.2%; dissolved in DMSO) was tested in a test for HPRT locus mutation in mammalian cells in vitro in Chinese hamster V79 cells. The study complied with test guideline OECD 476. Six concentrations of up to 500 µg/ml were used in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). No cytotoxicity was observed at concentrations up to 1000 µg/ml; precipitation of the test material was seen at concentrations of 500 µg/ml and greater. Cells were exposed to thiacloprid for 5 h and cultured for an additional 4 days and 7 days before subculturing in selective medium. Duplicate cultures were used at each concentration and results were confirmed in independently repeated assays. There was no significant dose-related or reproducible increase in the frequency of mutants above that of the negative controls. The positive controls (ethylmethanesulfonate and dimethylbenzanthracene) produced significant increases in the frequency of mutants (Brendler-Schwaab, 1996a).

The clastogenic potential of thiacloprid (purity, 96.8–97.2%; dissolved in DMSO) was tested in a test chromosome aberration in Chinese hamster V79 cells in vitro. The study complied with test guideline OECD 473. Cells were exposed for 4 h to thiacloprid at a concentration of 0, 75, 300 or 750 µg/ml in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver

S9). Duplicate cultures were used at each concentration. Cells were harvested at 18 h (all concentrations) and at 30 h (0 and 750 µg/ml), and 100 metaphases from each of two parallel cultures were examined for chromosome aberrations. Cytotoxicity was seen at the highest concentration used in this assay. No statistically significant or biologically relevant increase in the number of cells with chromosome aberrations was seen at any concentration. The positive controls (mitomycin C and cyclophosphamide) produced a significant increase in the number of cells with chromosome aberrations (Herbold, 1995b).

The potential of thiacloprid (purity, 97.2%; dissolved in DMSO) to induce unscheduled DNA synthesis (UDS) in mammalian cells in vitro was tested in freshly prepared cultures of Sprague-Dawley rat hepatocytes. The study complied with test guideline OECD 482. Cells were treated with thiacloprid for 22 h at seven concentrations ranging from 75 to 500 µg/ml in the presence of ^{3}H-thymidine at 10 µCi/ml (13.6 Ci/mmol). Cytotoxicity was seen at a concentration of 450 and 500 µg/ml (69.8% or 45.5% survival, respectively). After autoradiography, 150 cells per concentration were scored for nuclear and cytoplasmic grains. No statistically significant increase in the number of net nuclear grains was seen at any concentration. The positive control (2-acetylaminofluorene) produced a statistically significant increase in the number of net nuclear grains and the percentage of cells in repair (Brendler-Schwaab, 1996b).

In vivo

In an assay for induction of micronuclei, groups of 15 male and 15 female Hsd/Win:NMRI mice received a single intraperitoneal dose of thiacloprid (purity, 96.8–97.2%) at 60 mg/kg bw suspended in 0.5% aqueous Cremophor emulsion, while groups of five male and five female mice were used as vehicle controls and positive controls (cyclophosphamide; 20 mg/kg bw, intraperitoneal). The study complied with test guideline OECD 474. The femoral marrow of five mice of each sex treated with thiacloprid was prepared 16, 24 and 48 h after administration, while negative and positive controls were sacrificed 24 h after administration. One thousand polychromatic erythrocytes per animal were scored for micronuclei. The thiacloprid-treated mice displayed clinical signs (rough fur, apathy, spasms, difficulty in breathing) for up to 16 h, and three of the 40 animals died during the test period owing to the acute intraperitoneal toxicity of thiacloprid. The ratio between polychromatic and normochromatic erythrocytes was not changed by treatment with thiacloprid. No biologically or statistically significant increase in the number of micronucleated polychromatic erythrocytes was seen in thiacloprid treated mice. Clear evidence of clastogenicity was seen with the positive control (Herbold, 1995c).

Table 27. Results of studies of genotoxicity with thiacloprid

End-point	Test object	Concentration/dose	Purity (%)	Results	References
In vitro					
Reverse mutation	*S. typhimurium* (TA98, TA100, TA1535, TA1537)	± S9: 0, 16, 50, 158, 500, 1581, 5000 µg/plate	97.2	Negative	Herbold (1995a)
Reverse mutation	*S. typhimurium* (TA98, TA100, TA1535, TA1537) *E. coli* (WP2 *uvrA*)	± S9: 0, 313, 625, 1250, 2500, 5000 µg/plate	96.8	Negative	Ohta (1995)
Gene mutation	*Hprt* locus, Chinese hamster V79 cells	± S9: 0, 15.6, 31.3, 62.5, 125, 250, 500 µg/ml	96.8–97.2	Negative	Brendler-Schwaab (1996a)
Chromosomal aberration	Chinese hamster V79 cells	± S9: 0, 75, 300, 750 µg/ml	96.8–97.2	Negative	Herbold (1995b)
Unscheduled DNA synthesis (UDS)	Primary rat hepatocytes	0, 75, 150, 300, 350, 400, 450, 500 µg/ml	97.2	Negative	Brendler-Schwaab (1996b)
In vivo					
Micronucleus formation	Male and female NMRI mice, bone-marrow erythroblasts	0 and 60 mg/kg bw, single intraperitoneal dose	96.8–97.2	Negative	Herbold (1995c)

S9, 9000 × *g* supernatant from livers of male Sprague-Dawley rats.

2.5 Reproductive toxicity

(a) Multigeneration studies

In a range-finding study of reproductive toxicity, groups of seven male and seven female Charles River Crl:CD BR rats were given diets containing thiacloprid (purity, 98.6%) at a concentration of 0, 100, 400, or 1600 ppm for a minimum of 28 days before mating. Females were allowed to litter and were sacrificed after completion of lactation. The resulting F_1 litters were reduced to eight pups (four pups of each sex) on postnatal day 4. Three pups of each sex were sacrificed at postnatal day 21 and the remaining pups sacrificed at postnatal day 35. Histopathological examinations were conducted on the liver and thyroids from all parental and selected F_1 animals. Dietary analyses revealed satisfactory stability of the test material; dietary concentrations were within ± 15% of the nominal concentration. Mean daily intakes at 100, 400 and 1600 ppm were equivalent to about 7, 27 and 107 mg/kg bw per day.

One male at 1600 ppm was found dead on day 40, gross necropsy did not reveal the cause of death. No treatment-related clinical signs were noted in any animal. The parental body weights and feed consumption were consistently lower in both sexes at 1600 ppm before mating and in females at 1600 ppm during gestation, values occasionally attained statistical significance (Table 28).

Table 28. Relevant findings in a range-finding study of reproductive toxicity in rats fed diets containing thiacloprid

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	100	400	1600	0	100	400	1600
Body weight (g), before mating:								
Day 0	319.7	315.7	318.3	317.4	227.6	244.6	228.9	224.1
Day 28	431.9	419.6	433.6	384.3**	284.9	279.7	274.7	253.7**
Day 42	465.1	449.7	475.4	406.9*	—	—	—	—
Body-weight gain (g)	145.4	134.0	157.1	89.4**	57.3	55.1	45.9	29.6**
Body weight (g), gestation:								
Day 0	—	—	—	—	283.9	276.2	272.7	256.9**
Day 20	—	—	—	—	426.4	423.0	401.0	370.6*
Body-weight gain (g)	—	—	—	—	142.6	146.8	128.3	113.7
Body weight (g), lactation: day 0								
Day 0	—	—	—	—	325.7	325.0	309.3	290.7*
Day 21	—	—	—	—	343.0	351.6	341.5	338.0
Body-weight gain (g)	—	—	—	—	17.3	26.6	32.2	47.3**
Microscopic findings, F_0								
Hepatocyte hypertrophy	0/7	0/7	0/7	7/7	0/7	0/7	1/7	7/7
Hepatocyte, glycogenic vacuolar change	4/7	5/7	4/7	0/7	1/7	1/7	2/7	0/7
"Ground glass" hepatocytes	0/7	0/7	0/7	6/7	0/7	0/7	0/7	5/7
Hepatocyte cytoplasmic inclusions	0/7	0/7	0/7	0/7	0/7	0/7	0/7	3/7
Thyroid follicular hypertrophy	0/7	0/7	1/7	4/7	0/7	0/7	0/7	6/7
Microscopic findings, F_1								
Hepatocyte hypertrophy	0/7	0/5	0/6	6/7	07	0/5	0/6	7/7
Hepatocyte, glycogenic vacuolar change	6/6	2/5	3/6	0/7	5/6	3/5	3/6	0/7

"Ground glass" hepatocytes	07	0/5	0/6	7/7	07	0/5	0/6	7/7
Hepatocyte cytoplasmic inclusions	0/7	0/5	0/6	1/7	0/7	0/7	0/7	0/7
Increased mitotic figures (liver)	0/7	0/5	0/6	4/7	0/7	0/5	0/6	4/7
Thyroid follicular hypertrophy	0/7	0/5	0/6	2/7	0/7	0/5	0/6	1/7

From Porter et al. (1995)
* $p < 0.05$; ** $p < 0.01$.

Table 29. Findings related to reproductive toxicity in a range-finding study in female rats fed diets containing thiacloprid

Finding	Dietary concentration (ppm)			
	0	100	400	1600
Mating index	100	100	100	100
Fertility index	100	71.4	85.7	100
Gestation index	100	100	100	100
No. of litters	7	5	6	7
Total No. of pups born	107	73	81	97
Litter size, mean	15.3	14.6	13.5	13.9
No. of stillborn pups (%)	6 (5.6)	1 (1.4)	1 (1.2)	3 (3.1)
Live birth index (%)	95.0	98.8	99.0	96.8
Total No. of dead pups (%)	9 (8.4)	3 (4.1)	1 (1.2)	21* (21.6)
Pup deaths (day 0–4)	3	2	0	16
Viability index (%)	96.4	97.4	100.0	83.9
Pup deaths (days 5–21)	0	0	0	2
Weaning index, mean	100	100	100	96.4
Mean weight of viable pups (g), birth	6.0	6.3	6.6	6.0
Day 4	9.8	10.4	10.4	8.1*
Day 11	26.0	27.5	26.6	19.3**
Day 21	56.1	57.1	53.6	41.0**
Day 28	93.3	92.7	90.2	66.8**
Day 35	141.8	140.2	133.7	104.0**
Body-weight gain (g)	135.8	133.8	127.1	98.1**

From Porter et al., (1995)
* $p < 0.05$; ** $p < 0.01$.

Histopathological effects in the liver (hepatocyte hypertrophy, "ground glass" appearance of hepatocyte cytoplasm, hepatocyte glycogenic vacuolar change) were seen at 1600 ppm and in one F_0 female at 400 ppm. Thyroid follicular cell hypertrophy was also noted at 1600 ppm and in one F_0 male at 400 ppm. Similar findings were noted in F_0 and F_1 animals.

No treatment-related effects on reproductive performance were seen. The number of pup deaths (days 0–4) was significantly increased at 1600 ppm, resulting in a slightly lower viability index for this dose group. Mean pup weight at 1600 ppm was significantly lower from day 4, pup weights at birth were comparable in all dose groups (Table 29).

The NOAEL for parental toxicity was 400 ppm, equivalent to about 27 mg/kg bw per day, on the basis of microscopic hepatic and thyroid changes in F_0 and F_1 animals at 1600 ppm. The NOAEL for reproductive toxicity was 400 ppm, equivalent to about 27 mg/kg bw per day, on the basis of decreased body-weight gain and survival in pups at 1600 ppm (Porter et al., 1995).

In a two-generation study of reproductive toxicity, groups of 30 male and 30 female CD Sprague-Dawley rats were given diets containing thiacloprid (purity, 96.7–97.5%) at a concentration of 0, 50, 300 or 600 ppm. Animals were mated after 10 weeks to produce F_1 pups. After weaning, selected F_1 pups were given diets containing thiacloprid for 10 weeks before mating and production of the F_2 generation. The study complied with test guideline OECD 416. Determinations of body weights, feed consumption, clinical signs, estrous cycling, mating, fertility, duration of gestation and litter size were performed during the study. Offspring were examined for treatment-related effects on sex ratio, pup viability, body-weight gain and clinical signs. Gross necropsy evaluations were performed on all adults and pups. Histopathological examination of reproductive organs, liver, pituitary, thyroids and all gross lesions was performed on all F_0 and F_1 adults. Dietary analyses revealed satisfactory test substance stability and concentration. The mean daily intakes at 0, 50, 300 and 600 ppm in the period before mating were equal to 0, 3.5, 20.9 and 40.9 mg/kg bw per day in F_0 males and 0, 4.2, 26,0 and 50.8 mg/kg bw per day in F_0-females, respectively, and 0, 3.5, 21,7 and 43.9 mg/kg bw per day in F_1 males and 0, 4.1, 25.5 and 51.0 mg/kg bw per day in F_1 females, respectively.

Four F_0 females at 300 ppm and three females at 600 ppm were sacrificed or found dead on days 23–24 of gestation due to dystocia. These animals also exhibited pallor, perineal and vaginal staining. Small numbers of deaths in other groups are not attributable to the effects of treatment. No clinical signs of toxicity were noted in surviving animals.

In parental animals, body-weight gains were decreased at 600 ppm for F_0 and F_1 females during the phase before mating and for F_1 males. During the gestation phase lower body weights with normal weight gains were measured in the F_0 and F_1 groups at the highest dose. Also during the lactation phase lower body weights were observed in the parental and F_1 groups at the highest dose (Table 30). No consistent effect on feed consumption was seen in F_0 animals. Feed consumption was significantly increased in F_1 animals of both sexes at 600 ppm during the period before mating. No effects on feed consumption were seen during gestation in dams of either generation.

No effects were seen on fertility or reproductive performance. Gestation duration was increased in some F_0 animals at 300 and 600 ppm due to dystocia, however no significant effect was seen on the mean duration of gestation for these groups (Table 31).

There was a possible treatment-related decrease in the live birth index at 600 ppm for the F_1 and F_2 generations when compared with the concurrent controls and also with the historical controls (97–100%; data from 10 two-generation studies). The viability index was statistically non-significantly but markedly lower in the F_1 animals at 600 ppm (97 and 83 for the control group and the group at the highest dose, respectively). However, the low viability index at 600 ppm was due to the cannibalization of pups. The viability index for the control group and the group at the highest dose was 99 and 91, respectively, when the index was calculated without including the cannibalized pups as part of the litter on lactation day 0. The viability index of 91 was not considered a compound-related reduction in pup viability as the viability index for the F_2 generation control group was 94 and the historical-control range for the viability index was 91–100. There were a markedly higher number of cannibalized pups at 600 ppm when compared with the control group; however, when the litter was used as the experimental unit, no statistically significant difference was found for the number of dams with cannibalized pups.

The pup weights were decreased in the F_1 and F_2 groups at the intermediate and highest doses on days 14–21, and days 7–21, respectively.

Table 30. Relevant findings in a two-generation study of reproductive toxicity in rats fed diets containing thiacloprid

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	50	300	600	0	50	300	600
F_0 generation								
Body weight (g), before mating:								
Day 0	219.0	217.4	221.9	222.6	148.3	148.5	150.6	151.4
Day 70	395.4	395.5	397.4	392.2	229.8	228.0	220.9	218.4*
Body-weight gain	176.4	178.1	175.3	169.6	81.5	79.5	70.3	67.0
Body weight (g), gestation:								
Day 0	—	—	—	—	232.8	231.7	222.1*	220.9*
Day 20	—	—	—	—	356.9	361.2	351.5	337.4**
Body-weight gain (g)	—	—	—	—	124.1	129.6	129.3	116.5
Body weight (g), lactation:								
Day 0	—	—	—	—	271.1	273.3	264.4	252.6*
Day 21	—	—	—	—	307.8	304.6	297.4	287.3**
Body-weight gain (g)	—	—	—	—	36.7	31.3	33.0	34.7
Liver weight (g)	17.4	18.5	20.4*	22.4*	16.6	16.9	17.4	19.7*
Relative liver weight (g/100g)	4.29	4.47	4.94*	5.50*	5.71	5.86	6.19	7.02*
Thyroid weight (g)	0.020	0.020	0.022	0.025*	0.014	0.014	0.016*	0.017*
Relative thyroid weight (g/100g)	0.0049	0.0049	0.0055	0.0061*	0.0047	0.0049	0.0058*	0.0059*
Hepatocyte hypertrophy	0/30	0/30	10/29*	28/30*	0/30	0/30	10/30*	26/30*
Severity[a]	—	—	1.0	1.5	—	—	1.0	1.8
Follicular hypertrophy	5/30	4/30	7/29*	20/30*	0/30	0/30	5/30*	17/30*
Severity[a]	1.0	1.0	1.0	1.0	—	—	1.0	1.0
F_1-generation								
Body weight (g), before mating, day 0	216.6	208.0	213.3	177.9**	157.6	156.1	160.0	145.1**
Day 70	410.1	393.0	403.6	351.3**	242.3	249.4	237.1	222.2**
Weight gain	193.5	185.0	190.3	173.4	84.7	93.3	77.1	77.1
Body weight (g), gestation:								
Day 0	—	—	—	—	244.0	249.2	236.0	223.1**
Day 20	—	—	—	—	364.8	371.5	353.0	337.8*
Body-weight gain (g)	—	—	—	—	120.9	122.3	117.0	114.6
Body weight (g), lactation:								
Day 0	—	—	—	—	277.0	284.6	268.3	252.8**
Day 21	—	—	—	—	307.6	312.3	304.5	291.0**
Body-weight gain (g)	—	—	—	—	30.6	27.7	36.2	38.2
Liver weight (g)	20.3	18.9	20.9	20.6	16.2	17.8	18.8*	20.9*
Relative liver weight (g/100g)	4.52	4.46	4.84	5.43*	5.54	5.95	6.54*	7.55*
Thyroid weight (g)	0.022	0.021	0.025	0.023	0.016	0.017	0.017	0.018
Relative thyroid weight (g/100g)	0.0050	0.0050	0.0058*	0.0061*	0.0056	0.0057	0.0060	0.0065*
Gonads weight, relative	0.876	0.918	0.936	1.071*	0.043	0.047	0.046	0.049*
Hepatocyte hypertrophy	0/30	0/29	18/30*	27/30*	0/30	0/30	16/30*	29/30*
Severity[a]	—	—	1.1	1.8	—	—	1.0	1.9
Follicular hypertrophy	6/30	7/29	13/30	19/30*	4/30	4/30	18/30*	25/30*
Severity[a]	1.0	1.0	1.0	1.2	1.0	1.0	1.0	1.1

From Eigenberg & Hamilton (1997)

In dams that died or were sacrificed due to dystocia, red foci were detected in the liver. No gross treatment-related effects were noted at necropsy of surviving adults or pups. Relative and absolute liver weights were significantly increased in adult males and females of both generations at ≥ 300 ppm. Relative and absolute F_0 thyroid weights were significantly increased at 600 ppm in both sexes and in females only at 300 ppm. Relative thyroid weight was increased in F_1 females only at 600 ppm. Relative ovary and testes weights were slightly (but significantly) increased in F_1 adults at 600 ppm (Table 30).

Microscopy revealed an increased incidence and severity of hepatocyte and thyroid follicular cell hypertrophy in both sexes at ≥ 300 ppm. The severity and incidence of these findings was also noted to be greater in F_1 than F_0 animals. Hepatocellular necrosis was noted in the livers of dams sacrificed or found dead. No further changes attributable to treatment were noted in adults. Pups were not examined microscopically.

Table 31. Findings related to reproductive toxicity in a two-generation study in rats fed diets containing thiacloprid

Finding	Dietary concentration (ppm)			
	0	50	300	600
F_0/F_1 generation				
No. of animals mated	30	29	30	30
Mating index (%)	100	100	100	100
Fertility index (%)	93.3	100.0	93.3	100.0
Gestation index (%)	100.0	100.0	82.1	90.0
Gestation length [range] (days)	22.2 [21–23]	22.3 [21–23]	22.4 [21–25]	22.4 [21–24]
No. of litters	28	29	24	27
No. of cannibalized. pups / litters with cannibalized. pups	5/4	11/6	6/4	17/7
No. of pups born (litter size)	314 (11.2)	360 (12.4)	290 (12.1)	282 (10.4)
No. of liveborn pups (litters with liveborn pups)	311 (28)	344 (29)	275 (23)	262 (27)
No. of stillborn pups (%)	2 (0.6)	16 (4.4)	13 (4.5)	16 (5.7)
Live birth index	99.1	95.8	94.9	91.0
Viability index	97.4	91.5	89.9	82.8
Lactation index	99.6	96.6	98.8	99.5
Mean weight of viable pups (g), male/female:				
Birth	6.8/6.4	6.9/6.5	6.7/6.3	6.6/6.3
Day 4	10.4/9.9	10.2/9.7	10.0/9.6	9.7/9.3
Day 7	15.7/15.2	15.8/15.3	15.3/14.8	14.3*/13.9*
Day 14	30.0/29.4	30.0/29.2	27.9/27.0*	25.8**/25.2**
Day 21	48.5/47.5	49.5/47.8	44.6/43.1*	41.2**/40.7**
No. of "weak" pups (litters)	1 (1)	18 (3)	5 (2)	1 (1)
F_1/F_2 generation				
No. of animals mated	30	30	30	30
Mating index (%)	100	100	100	100
Fertility index (%)	86.7	93.3	86.7	93.3
Gestation index (%)	96.2	100	100	96.4
Gestation length [range] (days)	22.3 [21–23]	22.3 [21–24]	22.3 [22–23]	22.2 [20–23]

No. of litters	25	28	26	28
No. of cannibalized. pups / litters with cannibalized. pups	3/3	2/1	7/5	14/6
No. of pups born (litter size)	306 (12.2)	347 (12.4)	318 (12.2)	313 (11.2)
No. of liveborn pups (litters with liveborn pups)	297 (25)	331 (28)	308 (26)	292 (27)
No. of stillborn pups (%)	9 (2.9)	14 (4.0)	8 (2.5)	18 (5.8)
Live birth index	95.8	95.9	96.6	90.2
Viability index	93.9	98.1	94.5	91.6
Lactation index	99.0	99.6	98.1	98.1
Mean weight of viable pups [g], male/female				
Birth	6.8/6.4	6.8/6.4	6.8/6.4	6.5/6.2
Day 4	10.2/10.0	10.7/10.2	10.0/9.4	9.5/9.1
Day 7	15.8/15.4	16.4/15.7	15.2/14.5	14.2*/13.6**
Day 14	30.9/30.1	31.9/30.4	28.5**/27.4**	26.3**/25.5**
Day 21	51.1/49.2	50.5/48.4	46.6*/44.7*	41.2**/39.3**
No. of "weak" pups (litters)	1 (1)	14 (5)	18 (5)	16 (5)

From Eigenberg & Hamilton (1997)

* $p < 0.05$; ** $p < 0.01$.

The NOAEL for parental toxicity was 50 ppm, equal to 3.5 and 4.2 mg/kg bw in males and females, respectively, on the basis of liver and thyroid effects (increased weights, hepatocyte hypertrophy, thyroid follicular cell hypertrophy) at 300 ppm and greater. The NOAEL for reproductive toxicity was 50 ppm on the basis of dystocia and decreased pup weights at 300 ppm and greater and decreased live birth index at 600 ppm (Eigenberg & Hamilton, 1997).

(b) Developmental toxicity

Rats

In a study of prenatal developmental toxicity, groups of 35 (initially 28) inseminated Wistar (Hsd Cpb:WU) rats were given thiacloprid (purity, 97.0–97.3%) at a dose of 0, 2, 10 or 50 mg/kg bw per day, by gavage in 0.5% carboxymethylcellulose on postcoitum days 6–19. The study complied with test guideline OECD 414. The dose levels were based on the results of a pilot study of developmental toxicity in rats given doses of 0, 2, 10, and 50 mg/kg bw per day. At the highest dose, maternal toxicity (reduced body-weight gain and feed intake, increased water intake and increased micturition), reduced fetal weights and an increased incidence of skeletal retardations were noted. The NOAEL in the pilot study was 10 mg/kg bw per day.

Fetuses were delivered by caesarean section on day 20 of gestation, and dams were subjected to gross pathological investigation after sacrifice on day 20. Approximately half of the fetuses were examined by serial sectioning and half were differentially stained for skeletal examination. Owing to inadequate skeletal and cartilage staining for unknown reasons in single fetuses from several litters of the different dose groups (22, 23, 23 and 43 fetuses from four, four, four, and nine litters at 0, 2, 10 and 50 mg/kg bw per day, respectively) seven additional inseminated females were allocated non-randomly to the study at each dose in order to obtain a minimum of 20 litters for complete skeletal and cartilage evaluation in this study. Except for the data from skeletal and cartilage examination, all parameters determined for the additionally assigned animals were processed and evaluated together with the animals originally allocated to the study (total of 35 inseminated females per dose group). In the case of skeletal and cartilage examination the data generated were evaluated and reported separately for completely evaluated litters and for litters where complete evaluation was not possible due to insufficient staining.

No clinical signs or increased mortalities were detected at any dose; the appearance and behaviour of the dams was unaffected by treatment up to and including 50 mg/kg bw per day. Feed consumption of the dams was distinctly decreased, especially during the first few days after initiation of treatment at 50 mg/kg bw, leading to body-weight losses during the first few days after initiation of treatment and to a reduction of total body-weight gain, so that cumulative and corrected body-weight gains during gestation were significantly decreased at 50 mg/kg bw per day (Table 32). Water consumption and the amount of excreted faeces were decreased during the first few days after initiation of treatment at 50 mg/kg bw per day, while increased water consumption and urine excretion were observed towards and during the second half of gestation at the highest dose (50 mg/kg bw per day). At gross necropsy no test compound-related gross pathological findings were evident in any of the experimental groups.

At caesarean section, an increased incidence of late resorptions and one total resorption were observed at 50 mg/kg bw per day, resulting in a slightly decreased number of live fetuses and mean litter size at the highest dose. Also, mean fetal weight was decreased at the highest dose. (Table 32). There was no effect on placental weight. An increased incidence of placental border necrosis was apparent in all dose groups; however, values were within the range for historical controls for the laboratory (0–17.65% of placentas with findings; 0–42.86% of litters with findings).

The external examination of the fetuses revealed forelimb deviations in two fetuses from a single litter at 50 mg/kg bw per day. These limb findings were considered to be treatment-related since they were confirmed by skeletal evaluation (bone dysplasia) and similar findings were observed at 50 mg/kg bw per day in other fetuses in several litters which were incompletely evaluated.

Visceral examination of fetuses revealed an apparent increase in the incidence and severity of renal pelvis dilatation in groups at 10 mg/kg bw per day and greater. Since the combined incidence of slight renal pelvis dilatation and renal pelvis dilatation did not display dose-dependency and in the group at the highest dose was only marginally greater than (fetal incidence) or less than (litter incidence) the control value for a study of a developmental toxicity with comparable study design (fetal incidence, 23.2%; litter incidence, 69.2%), the finding was not considered to be related to treatment.

Table 32. Relevant findings in a study of developmental toxicity in rats given thiacloprid by gavage

Finding	Dose (mg/kg bw per day)			
	0	2	10	50
Body weight (g):				
Day 6	228.4	231.6	228.4	230.5
Day 9	236.0	238.8	234.2	212.8**
Day 14	255.6	256.9	252.8	232.2**
Day 20	314.7	314.1	308.8	279.6**
Bosy-weight gain (g), days 6–19	77.6	74.0	71.7	42.0**
Feed consumption (g/animal per day):				
Days 6–11	17.7	17.6	16.9	6.4**
Days 11–16	19.1	19.1	19.0	16.4**
Days 16–20	21.6	21.4	20.9	19.6**
No. of dams with implantations (a)	28	31	32	30
No. of dams with viable fetuses (b)	28	31	32	29
No. of implantations per dam (b)	12.4	11.5	11.4	11.8
% Implantations per dam (b)	91.1	92.5	95.7	79.3**
% Postimplantation loss per dam (a)	0.9	0.9	0.5	2.8*

% Postimplantation loss per dam (b)	0.9	0.9	0.5	2.5
% Late resorptions per dam (a)	0.9	0.8	0.5	2.8
% Late resorptions per dam (b)	0.9	0.8	0.5	2.4
No. of live fetuses (b)	321	329	348	270
Mean No. live fetuses per litter (b)	11.5	10.6	10.9	9.3
% Males (b)	51.3	53.1	56	47.6
Fetal weight [g] (b)	3.70	3.73	3.69	3.20**
Placental weight [g] (b)	0.60	0.64	0.60	0.60
Necrotic placental border, No. of placentas (%)	12 (3.7)	35 (10.6)	42 (12.1)	39 (14.4)
No. of litters (%)	6 (21.4)	10 (32.3)	8 (25.0)	13 (44.8)

From Stahl (1997)

* $p < 0.05$; ** $p < 0.01$.

Note: In one dam at 50 mg/kg bw per day, total resorption of the conceptus was observed. For this group, the No. of dams with implantations was 30 while the No. of dams with viable fetuses was 29. For all other groups, the No. of dams with implantations and with viable fetuses was identical. Since total resorptions can also occur by chance, the percentages of postimplantation loss per dam and of late resorptions per dam were given for dams with implantations (a) and for dams with viable fetuses (b). For all other parameters in this table, the values were given only for dams with viable fetuses (b).

The skeletal evaluation revealed an increased incidence of reduced ossification in fetuses of the group at the highest dose, mainly at the following locations: distal and proximal phalanges, metacarpals, sternebrae, cervical, thoracic and caudal vertebral bodies, caudal verterbal arch, fontanelle (enlarged) as well as parietal, interparietal and supraoccipital bone. Also, skeletal variations (wavy ribs, asymmetrical vertebrae) were significantly increased at 50 mg/kg bw per day (Table 33). The incidence of some findings was also increased at 10 mg/kg bw per day; however, these findings were not considered to be of toxicological relevance since dose-dependency was lacking and all values were within the range for historical controls.

Regarding malformations, an increased incidence of fetuses or litters with limb bones dysplasia together with one fetus with multiple malformations (gastrochisis, shortened mandible, cleft palate, limb deviations, etc.) was observed at 50 mg/kg bw per day. Dysplasia of limb bones in the group at the highest dose comprised findings of the clavicula (thickened, constricted, kinked), scapula (shortened, kinked), humerus (shortened, thickened, bent, kinked), radius (shortened, thickened, kinked), ulna (shortened, thickened, kinked), femur (shortened, thickened, kinked), tibia (kinked) and fibula (kinked). The increased total incidence of malformations at the highest dose was attributable to the increased incidence of limb bones dysplasia and the multiple malformations in one fetus (Table 33).

The incidence of fetuses or litters with dysplasia of limb bones was increased in the group at the highest dose when compared with the control groups and the groups at the lowest and the intermediate doses. At 50 mg/kg bw per day, eight fetuses (5.71% of the fetuses assigned to skeletal evaluation) in six litters (20.69%) were affected. Data from another study of developmental toxicity in rats treated orally which was started 6 months later in the same laboratory (T2060240, January to March 1996) revealed 10 fetuses (6.58% of the fetuses assigned to skeletal evaluation) with dysplasia of limb bones in six litters (23.08%) of the control group. Data from a further recent oral developmental toxicity study (T5068551, April to June 2000) showed dysplasia of limb bones in 10 fetuses (8.13% of the fetuses assigned to skeletal evaluation) in four litters (20.0%) of the control group. Thus, the incidence of fetuses and litters with dysplasia of limb bones in the group at 50 mg/kg bw per day was within the normal range of scattering for the strain of rats used and the type of these findings in the group at 50 mg/kg bw per day was comparable to findings occurring spontaneously in the strain of rats used (Holzum, 2000).

Table 33. Selected variations and malformations in a study of developmental toxicity in rats given thiacloprid by gavage

Finding	Dose (mg/kg bw per day)			
	0	2	10	50
Slight dilatation of renal pelvis, No. of fetuses / %	22/14.1	15/9.5	32/18.9	18/13.8
No. of litters / %	13/46.4	9/29.0	19/59.4	14/48.3
Dilatation of renal pelvis, No.of fetuses / %	3/1.9	6/3.8	15/8.9	15/11.5
No. of litters / %	3/10.7	5/16.1	9/28.1	10/34.5
Total dilatation of renal pelvis, No.of fetuses / %	25/16.0	21/13.3	47/27.8	33/25.4
No. of litters / %	14/50.0	13/41.9	23/71.9	18/62.1
Incomplete ossification 2nd sternebra; No.of fetuses / %	53/37.1	70/47.3	96/61.5**	66/68.0**
No. of litters / %	22/91.7	725/92.6	27/96.4	19/95.0
Incomplete ossification 3rd sternebra; No.of fetuses / %	2/1.4	9/6.1	11/7.1	7.1/17**
No. of litters / %	2/8.3	7/25.9	7/25	11/55.0**
Incomplete ossification. 4th sternebra; No.of fetuses / %	10/7.0	11/7.4	18/11.5	17/17.5*
No. of litters / %	7/29.2	9/33.3	12/42.9	12/60.0
Incomplete ossification 5th sternebra; No.of fetuses / %	35/24.5	43/29.1	54/34.6	38/39.2
No. of litters / %	18/75.0	15/55.6	21/75.0	17/85.0
Asymmetrical 3rd sternebra; No.of fetuses / %	5/3.5	7/4.7	15/9.6	15/15.5**
No. of litters / %	5/20.8	6/22.2	10/35.	11/55.0
Asymmetrical 4th sternebra; No.of fetuses / %	6/4.2	16/10.8	22/14.1*	17/17.5**
No. of litters / %	6/25.0	12/44.4	12/42.9	12/60.0%
Wavy ribs (total); No.of fetuses / %	15/10.5	7/4.7	8/5.1	25/25.8**
No. of litters / %	8/33.3	6/22.2	5/17.9	13/65.0
Enlarged fontanelle; No.of fetuses / %	6/4.2	8/5.4	15/9.6	40/41.2**
No. of litters / %	5/20.8	5/18.5	11/39.3	17/85.0**
Malformations				
Multiple malformation; fetuses/litters	0	0	0	1
Microphthalmia, anophthalmia; fetuses/litters	5/5	2/2	2/2	7/6
Situs inversus totalis, fetuses/litters	0	0	1	0
Brain malformation; fetuses/litters	1	0	0	0
Malformation of ribs; fetuses/litters	0	0	1	0
Malformation of vertebra; fetuses/litters	0	3/3	1	1
Pelvis shift; fetuses/litters	0	2/2	0	0
Dysplasia of limb bones; fetuses/litters	1	2/2	0	8*/6
Deviation of hindlimbs and tail; fetuses/litters	1	0	0	0
No of fetuses with malformations (%)	7 (2.2)	6 (1.8)	5 (1.4)	17* (6.3)
No of litters with malformations (%)	7 (25)	5 (16.1)	4 (12.5)	12 (41.4)

From Stahl (1997)

* $p < 0.05$; ** $p < 0.01$.

There was no evidence for a specific potential for developmental toxicity with thiacloprid at up to and including the highest dose tested (50 mg/kg bw per day) since loss after implantation, decreased fetal weight as well as all morphological alterations of embryonic or fetal development observed in this study were manifest only at doses that also induced severe maternal toxicity and since the type of malformations observed at the highest dose were common spontaneous malformations in this rat strain.

Thiacloprid was not teratogenic in this study of prenatal developmental toxicity in rats. The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of decreased body weights and feed intake at 50 mg/kg bw per day. The NOAEL for developmental toxicity was 10 mg/kg bw per day on the basis of increased resorptions, reduced fetal weights, and an increase in skeletal variations at 50 mg/kg bw per day (Stahl, 1997).

Rabbits

In a study of prenatal developmental toxicity, groups of 24 inseminated female Himalayan (CHBB:HM) rabbits were given thiacloprid (purity, 97.3%) at a dose of 0, 2, 10 or 45 mg/kg bw per day by gavage, in 0.5% carboxymethylcellulose on postcoitum days 6–28. The study complied with test guideline OECD 414. The doses were based on the results of a pilot study in which doses of 0, 3, 10, 30, 40, and 50 mg/kg bw per day were tested. Maternal toxicity, such as reduced body-weight gain and feed intake, decreased water intake and decreased micturiton was observed at 30 mg/kg bw and higher doses. At doses of greater than 50 mg/kg bw per day, the resorption rate was increased, the number of fetuses and the fetal weights were reduced, and an increased incidence of arthrogryposis and skeletal variations and retardations was detected. Owing to an unclear dose–response relationship, a second study with doses of 0, 1, and 10 mg/kg bw per day was conducted which had no effects and in which the NOAEL was 10 mg/kg bw.

Fetuses were delivered by caesarean section on day 29 and were examined by evisceration and for skeletal abnormalities by Alcian Blue/Alizarin Red differential staining.

Two rabbits at 45 mg/kg bw per day aborted on day 23 or 28, and one at 2 mg/kg bw per day on day 24. Except for these abortions, there were no further effects on appearance and behaviour at doses up to and including 45 mg/kg bw per day. The feed intakes of the females were decreased at levels of 10 mg/kg bw per day and greater (severely at 45 mg/kg bw per day) (Table 34), and water intakes were decreased at 45 mg/kg bw per day. Correlating with decreased feed and water intake, reduced production of faeces and urine was noted in the majority of animals at the highest dose. The females lost weight during the first week of treatment at 10 mg/kg bw per day and greater (distinctly at 45 mg/kg bw per day), which resulted in decreased weight gains during treatment and gestation. Gross necropsy of the females revealed findings in the gastrointestinal tract (hardened contents in the stomach, marked vascular pattern of the intestines) at 45 mg/kg bw per day.

With respect to intrauterine development, the rate of gestation was decreased at 45 mg/kg bw per day by two abortions and three total resorptions (Table 34). Loss after implantation was increased at 45 mg/kg bw per day (statistically significantly when calculated per group only) owing to total resorption experienced by three females in this group. When loss after implantation was calculated excluding these females with total resorption it was unaffected by treatment at levels up to and including 45 mg/kg bw per day and so, correspondingly, was the number of fetuses. The fetal weight was statistically significantly decreased at 45 mg/kg bw per day when calculated on a litter and on an individual basis. At 10 mg/kg bw per day, fetal weight was only marginally decreased and statistical significance was only achieved when calculated on an individual basis but not when calculated on a litter basis. The number of male fetuses was significantly lower at the highest dose. However, the lower percentage of males was within the range for historical controls and therefore considered to be incidental. The placental weight was marginally decreased at 45 mg/kg bw per day.

Table 34. Relevant findings in a study of developmental toxicity in rabbits given thiacloprid by gavage

Finding	Dose (mg/kg bw per day)			
	0	2	10	45
Feed consumption (g/animal per day):				
Days 6–11	72.5	76.7	52.1**	17.1**
Days 11–16	59.3	70.3	49.5	29.2**

Days 16–21	73.3	78.7	62.5	44.3**
Body weight (g):				
Day 6	2573.8	2571.9	2588.9	2553.6
Day 13	2571.6	2568.9	2547.0	2427.6
Day 29	2741.8	2779.2	2725.5	2571.4**
Weight gain (g), days 6–11	–17.1	–20.2	–51.8*	–113.0**
Weight gain (g), days 6–28	154.5	191.4	122.5	5.4**
Gravid uterus weight (g)	350.8	340.6	348.1	252.6**
Corrected weight change (g), days 0–29	–179.4	–124.1	–214.3	–220.4
No. of females mated	24	24	24	24
No. of dams with implantations (a)	22	21	24	22
No. of dams with viable fetuses (b)	22	20	24	19
Abortions	0	1	0	2
No. of total resorptions	0	1	0	3
No. of implantations per group (a)	171	155	180	154
No. of implantations per group (b)	171	147	180	133
No. of implantations per dam (a)	7.8	7.4	7.5	7.0
No. of implantations per dam (b)	7.8	7.3	7.5	7.0
Postimplantation loss (a)	20	23	8	40**
Postimplantation loss (b)	20	15	8	19
% Postimplantation loss per dam (a)	0.9	1.1	0.3	1.8
% Postimplantation loss per dam (b)	0.9	0.8	0.3	1.0
Late resorptions per group (a)	20	23	8	32
Late resorptions per group (b)	20	15	8	19
% Late resorptions per dam (a)	0.9	1.1	0.3	1.5
% Late resorptions per dam (b)	0.9	0.8	0.3	1.0
No. of live fetuses (b)	151	132	172	114
Mean No. live fetuses/litter (b)	6.9	6.6	7.2	6.0
% Male fetuses (b)	51.4	42.6	44.5	35.5**
Fetal weight (g), litter basis	35.81	36.69	33.58	28.57**
Fetal weight (g), individual basis	35.10	35.25	32.94**	27.51**
Placental weight (g), litter basis	3.99	4.30	3.92	3.56
Placental weight (g), individual basis	3.91	4.01	3.83	3.37**

From Holzum (1996)

* $p < 0.05$; ** $p < 0.01$

Note: In one dam at 2 mg/kg bw per day, and in three dams at 45 mg/kg bw per day, total resorption of the conceptus was observed. Therefore the values for the selected parameters were given for dams with implantations (a) and for dams with viable fetuses (b).

The fetuses of the group at the highest dose revealed retarded ossification of different localizations (phalanges, metacarpals, calcanei, cervical and caudal vertebrae, hyoid bone) evident by statistically significant differences when calculated on a fetal basis and also for the phalanges when calculated on a litter basis (Table 35). Furthermore, the incidence of fetuses with supernumerary 13th ribs without supernumerary lumbar vertebra (variation) was marginally increased at 45 mg/kg bw per day. The difference did not achieve statistical significance. Nevertheless, a relationship to treatment cannot be excluded for this finding as two additional fetuses at 45 mg/kg bw per day revealed supernumerary 13th ribs combined with a supernumerary lumbar vertebra.

A slight increase in the number of malformed fetuses, largely attributable to an increased incidence of forelimb arthrogryposis was seen at 45 mg/kg bw per day (Table 35). Arthrogryposis was reported to be the most frequent spontaneous malformation in the strain of rabbits used. The incidence of fetuses with arthrogryposis in the group at the highest dose (4.4%) lay within the upper range for historical controls (5.6%).

There was no evidence for a specific potential for developmental toxicity with thiacloprid up to and including the highest dose tested (45 mg/kg bw per day) since loss after implantation, decreased fetal weight as well as all morphological alterations of embryonic or fetal development observed in this study were manifest only at doses that also induced severe maternal toxicity and since the type of malformation observed at the highest dose was a common spontaneous malformation in this strain of rabbit.

Table 35. Selected variations and malformations in a study of developmental toxicity in rabbits given thiacloprid by gavage

Finding	Dose (mg/kg bw per day)			
	0	2	10	45
No. fetuses/litters examined	151/22	132/20	172/24	114/19
Thirteenth rib (variation); fetuses/litters (%)	0	1.5/10.0	0.6/4.2	3.5/15.8
Incomplete ossification				
Fifth right medial phalanx digit; fetuses/litters (%)	1.3/9.1	1.5/10.0	3.6/8.3	32.1**/84.2**
Fifth left medial phalanx digit; fetuses/litters (%)	2.6/9.1	1.5/10.0	3.6/8.3	34.2**/89.5**
Fifth right medial phalanx toe; fetuses/litters (%)	0	1.9/10.0	1.5/8.3	16.7**/42.1**
Fifth left medial phalanx toe; fetuses/litters (%)	0	0.9/5.0	2.3/8.3	13.2**/31.6*
First right metacarpal; fetuses/litters (%)	0	0.8/5.0	0.6/4.2	7.9**/21.1
First left metacarpal; fetuses/litters (%)	0	0.8/5.0	1.2/8.3	7.9**/21.1
Calcaneus, bilateral; fetuses/litters (%)	0	0	0	7.9**/21.1
First cervical vertebral body; fetuses/litters (%)	6.6/36.4	8.3/30.0	7.629.2	28.1**/57.9
Thirteenth caudal vertebral body; fetuses/litters (%)	4.0/18.2	0.8/5.0	1.2/8.3	15.2**/52.6
Hyoid body; fetuses/litters (%)	48.3/86.4	55.3/85.0	40.7/91.7	71.1**/89.5
Malformations				
Arthrogryposis; fetuses/litters	3/2	1	3/3	5/2
Small orbital cavity	1	0	0	0
Hydrocephalus internus	0	1	1	0
Cardiac septum defect	1	1	3/3	2/1
Missing kidney	0	0	0	1
Missing gall bladder	1	1	0	0
Fusion of ribs (cartilaginous part)	0	2/2	0	0
Supernumerary lumbar vertebra	0	1	0	0
Supernumerary lumbar vertebra with 13 rib(s)	0	0	0	2/2
No. of fetuses with malformations (%)	6 (4.0)	6 (4.6)	8 (4.7)	10 (8.8)
No. of litters with malformations (%)	4 (18.2)	5 (25.0)	7 (29.2)	6 (31.6)

From Holzum (1996)
* $p < 0.05$; ** $p < 0.01$.

Thiacloprid was not teratogenic in this study of prenatal developmental toxicity in rabbits. The NOAEL for maternal toxicity was 2 mg/kg bw per day on the basis of decreased body weights and feed intake at 10 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 2 mg/kg bw per day on the basis of reduced fetal weights at 10 mg/kg bw per day and greater, and a higher incidence of losses after implantation, supernumerary 13th ribs and incomplete ossification at 45 mg/kg bw per day (Holzum, 1996).

2.6 Special studies

(a) Neurotoxicity

In a study screening for acute neurotoxicity, groups of 12 male and 12 female fasted Fischer 344 rats were given thiacloprid (purity, 96.7–97.5%) as a single dose at nominal doses of 0, 20, 50 and 100 mg/kg bw (actual doses, 0, 22, 53 and 109 mg/kg bw) by oral gavage. The test substance was suspended in 0.5% methylcellulose/0.4% Tween 80 in deionized water and administered in a volume of 10 ml/kg bw. The study complied with the test guidelines of the US EPA.

Doses were selected on the basis of the results from a dose range-finding study of acute neurotoxicity, using analytically-confirmed doses of 27, 36, 85, 244 and 526 mg/kg bw for both sexes (five rats of each sex per dose). The NOAEL for clinical signs was 27 mg/kg bw for males and 36 mg/kg for females. In males, a dose of 36 mg/kg bw produced slight repetitive chewing movements. By comparison, a dose of 85 mg/kg bw produced slight repetitive chewing movements in both sexes, slight tremors in males and slight stains (oral and nasal) in one female each. Higher doses produced progressively more evidence of toxicity. The two highest doses of 244 and 526 mg/kg bw produced tremors, decreased activity, repetitive chewing movements, cool-to-touch body, dilated pupils, clear lacrimation, and 100% mortality in both sexes. The dose of 244 mg/kg bw also produced locomotor incoordination (ataxia) in one female. Clinical signs were generally apparent in both sexes within 2–4 h after treatment and recovered in survivors by the following day. Deaths (100%) at the two highest doses generally occurred within 24 h after dosing. The time of peak neurobehavioural effects (within the first 8 h after treatment) was estimated to be approximately 4 h after treatment.

In the main study, the following observations and measurements were performed: clinical observations, mortality, body weight, automated measurements of activity (figure-eight maze), a FOB, determination of brain weight, and a gross necropsy. Skeletal muscle, peripheral nerves, eyes (with optic nerves), and tissues from the central nervous system were examined histopathologically. Behavioural tests on treatment day 0 started at times of highest peak plasma concentrations.

Table 36. Relevant findings of a functional observational battery at day 0 in a study of acute neurotoxicity in rats given thiacloprid by gavage

Finding	Dose (mg/kg bw)							
	Males				Females			
	0	22	53	109	0	22	53	109
Home cage observations								
Gait incoordination: slight	0	0	0	6*	0	0	0	2
Tremors:								
Slight	0	0	0	3*	0	0	0	5*
Moderate to severe	0	0	0	7*	0	0	0	6*
Decreased activity	0	0	0	10*	0	0	0	12*
Ptosis of eyelids	0	0	0	7*	0	0	1	9*

Observations during handling								
Dilated pupils	0	0	0	9*	0	0	4*	11*
Open field observations								
Tremors:								
Slight	0	3	6*	0	0	0	3*	0
Moderate to severe	0	0	0	12*	0	0	0	12*
Gait incoordination:								
Slight	0	0	0	9*	0	0	0	8*
Moderate to severe	0	0	0	0	0	0	0	2*
Ptosis of eyelids	0	1+	1+	7+	0	0	0	10+
Reflex/physiological observations								
Approach response:								
No reaction	1	4	6*	10*	0	1	3*	11*
Slight reaction	11	8	6*	2*	12	11	9*	1*
Righting response:								
Slight incoordination	0	0	1	5*	0	1	1	9*
Landing on side	0	0	0	0	0	0	0	1*
Body temperature (°C)	36.6	36.7	36.6	32.3*	36.9	36.2	36.3	30.1*

From Sheets et al. (1997)
* $p < 0.05$; $^{+}$ not subjected to statistical analysis.

There were no deaths at any dose before scheduled terminal sacrifice. Clinical signs observed in both sexes after the highest dose were tremor, decreased activity, locomotor incoordination (ataxia), cool-to-touch body, dilated pupils, urine staining and eyelid ptosis. Further signs in females at the highest dose were red nasal and oral staining, clear nasal discharge, and clear lacrimation. At 53 mg/kg bw, only dilated pupils were seen in one female. The clinical signs occurred only on the day of treatment and were typically resolved by day 1, at the latest by day 4. Body weights were slightly reduced by treatment in males at the highest dose only.

In the FOB, compound-related effects were evident at the time of peak neurobehavioural effects on day 0 in males and females after all three doses (Table 36). Evidence of toxicity increased with dose, with minimal effects evident in only a few animals that received the lowest dose of 22 mg/kg bw and severe toxicity evident in all animals that received the highest dose of 109 mg/kg bw. To a large extent, the spurious difference in toxicity between the FOB and clinical observations reflects the rapid recovery after treatment, as clinical observations were performed some hours after the FOB and motor activity. All signs of toxicity resolved in all dosed groups by the next observation period on day 7.

Treatment-related decreases in motor and locomotor activity in the figure-eight maze were observed on day 0 in males at 109 mg/kg bw, and in females at all doses tested (Table 37). All signs were completely reversible in males and females by the next test occasion, 7 days after treatment. Habituation was not affected by treatment with thiacloprid.

Table 37. Motor and locomotor activity findings on day 0 in a study of acute neurotoxicity in rats given thiacloprid by gavage

Finding	Dose (mg/kg bw)							
	Males				Females			
	0	22	53	109	0	22	53	109
Motor activity (intervals 1 to 9)	205	249	169	89	514	375	302*	147*
Interval 1	140	147	111	16*	213	205	146*	33*
Interval 2	39	61	26	3*	147	90*	52*	11*
Interval 3	8	5	6	5	77	38	17*	22*
Locomotor activity (intervals 1 to 9)	70	83	58	3	162	103*	97*	2*
Interval 1	50	55	42	2*	74	62	46*	2*
Interval 2	13	18	9	0*	44	23*	17*	0*
Interval 3	3	2	3	0	22	9*	6*	0*

From Sheets et al. (1997)
* $p < 0.05$.

No compound-related gross lesions were detected in males or females at terminal sacrifice. Brain weight was not affected by treatment in males or females at any dose. Compound-related microscopic lesions were not seen in males or females at any dose.

The NOAEL was < 22 mg/kg bw for males and females on the basis of neurobehavioural effects at all doses. The NOAEL for microscopic lesions was 109 mg/kg bw for males and females, the highest dose tested (Sheets et al., 1997).

In a subsequent study of acute neurotoxicity, groups of 12 male and 12 female fasted Fischer 344 rats were given thiacloprid (purity, 96.8–97.0%) as a single dose at nominal doses of 0, 2.5 and 10 mg/kg bw (actual doses:, 0, 3.1 and 11 mg/kg bw) by oral gavage. The test substance was suspended in 0.5% methylcellulose/0.4% Tween 80 in deionized water and administered in a volume of 10 ml/kg bw. The study complied with the test guidelines of the US EPA. The following observations and measurements were performed in the study: clinical observations, mortality, automated measurements of activity (figure-eight maze) and FOB. Other parameters, like body weights, brain weight, gross necropsy, histopathology, were not included since a NOAEL was identified at higher doses in the aforementioned study. Also, no motor and locomotor activity (figure-eight maze) testing was performed in males since a NOAEL of 20 mg/kg bw had already been identified for this parameter in the aforementioned study. Behavioural tests at treatment day 0 started at times of highest peak plasma concentrations.

Table 38. Motor and locomotor activity findings at day 0 in a study of acute neurotoxicity in female rats given thiacloprid by gavage

Finding	Dose (mg/kg bw)		
	0	3.1	11
Motor activity (intervals 1 to 9)	447	442 (–1%)	351 (–21%)
Interval 1	227	206 (–9%)	184 (–19%)
Interval 2	119	111 (–7%)	86 (–28%)
Interval 3	58	51 (–12%)	37 (–36%)
Locomotor activity (intervals 1 to 9)	157	129 (–18%)	115 (–28%)
Interval 1	89	76 (–15%)	76 (–15%)
Interval 2	36	30 (–17%)	27 (–25%)
Interval 3	17	11 (–42%)	7 (–59%)

From Sheets (1997)

There were no deaths and no clinical signs at any dose before scheduled terminal sacrifice. In the FOB, no compound-related effects were detected at the time of peak neurobehavioural effects on day 0 in males and females at all three doses. For motor and locomotor activity, the pretreatment values for the groups that later received the test substance were generally within 20% of the control values. Therefore, differences that were less than 20% were considered to be within the range of normal variability in this laboratory for groups of 10–12 rats of each sex per dose and were not considered to be biologically significant. However, differences in motor and locomotor activity that exceeded 20% occurred at the highest dose of 11 mg/kg bw and were considered to be biologically significant (Table 38).

The NOAEL was 11 mg/kg bw for males on the basis of the neurobehavioural effects seen in the previous study at doses of 22 mg/kg bw and greater. The NOAEL was 3.1 mg/kg bw for females on the basis of decreases in motor and locomotor activity at 11 mg/kg bw (Sheets, 1997).

In a short-term study of neurotoxicity, groups of 12 male and 12 female Fischer 344 rats were fed diets containing thiacloprid (purity, 96.6–97.5%) at a nominal concentration of 0, 50, 400 or 1600 ppm for 13 weeks. The study complied with the test guidelines of the US EPA. Clinical observations, mortality, body weight and feed consumption were recorded at suitable time-points. An FOB of tests and automated measurements of activity in a figure-eight maze were evaluated before the start of the study and during weeks 4, 8 and 13. Ophthalmological examinations were performed before the start of treatment and during week 12. All rats received a gross examination at necropsy. The brain was removed and weighed. Skeletal muscle, peripheral nerves, eyes (with optic nerves) and tissues from the central nervous system were examined microscopically (six of each sex per dose from the control grop and group receiving the highest dose). Analysis of the diet indicated that the homogeneity and stability of the test material were acceptable and that the actual dietary concentrations were 0, 43.3, 357 and 1527 ppm. The mean daily intakes were equivalent to 0, 2.94, 24.2 and 101 mg/kg bw per day in males, and 0, 3.41, 27.9 and 115 mg/kg bw per day in females, at the nominal doses of 0, 50, 400 and 1600 ppm, respectively.

There were no deaths before terminal sacrifice, and no compound-related clinical signs were observed at any dietary level. Body weight was significantly reduced by 6–12% on day 7 in both sexes at 1600 ppm. In males, the body weight was significantly less than controls for most weeks of the study, while body weight in females recovered after the first week and was comparable to controls for the remainder of the study. The mean feed intake was reduced by 34–37% during the first week and by approximately 6–15% during many weeks of the remainder of the study at 1600 ppm (Table 39). Significant reductions in mean feed intake were also seen at 400 ppm in both sexes during the first week of the study (8–10% less). No treatment-related effects were detected by the FOB evaluations. Motor and locomotor activities were not affected by treatment. There were no treatment-related ophthalmological findings. Macroscopic and microscopic examinations did not detect any findings attributable to treatment. Brain weight was not affected by treatment.

Table 39. Relevant findings in a short-term study of neurotoxicity in rats given diets containing thiacloprid

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	50	400	1600	0	50	400	1600
Body weight (g):								
Day 0	194.0	194.2	193.4	196.0	131.3	131.1	131.5	131.8
Day 7	229.2	231.0	226.4	201.9*	146.1	145.7	146.1	137.4*
Day 91	359.5	363.1	356.0	335.6*	192.2	196.4	202.5	191.0
Feed consumption (g/animal per day):								
Day 7	20.25	19.94	18.26*	12.77*	13.55	13.17	12.40*	8.94*

Day 91	19.78	20.00	19.58	18.22*	13.10	13.09	13.40	12.40
Motor activity:								
Before treatment	523	601	584	619	795	953	907	880
Week 4	505	552	528	522	831	910	867	820
Week 8	595	504	537	500	834	988	971	928
Week 13	514	486	491	482	880	1147	1062	898
Locomotor activity:								
Before treatment	197	230	202	236	291	365	320	312
Week 4	181	196	187	183	267	339	278	270
Week 8	223	185	207	179	299	363	331	324
Week 13	227	208	211	196	298	411	365	319

From Sheets & Hamilton (1997)

* $p < 0.05$.

The NOAEL for systemic toxicity was 50 ppm, equal to 2.94 and 3.41 mg/kg bw per day in males and females, respectively, on the basis of reduced feed intake at 400 ppm and greater. The NOAEL for neurotoxicity was 1600 ppm, equal to 101 and 115 mg/kg bw per day in males and females, respectively, the highest dose tested (Sheets & Hamilton, 1997).

In a study of developmental neurotoxicity, groups of 25 female rats (strain: CRL:CD(SD)IGS VAF/PLUS) received diets containing thiacloprid (purity, 99.2 %) at a concentration of 0, 50, 300 or 500 ppm from day 0 of gestation to postnatal day 22. The study complied with the test guidelines of the US EPA. Analysis of the diet indicated that the homogeneity and stability of the test material were acceptable. The mean daily intakes for F_0 female rats at 0, 50, 300 and 500 ppm were equal to 0, 4.4, 25.6 and 40.8 mg/kg bw per day during gestation and 0, 8.2, 49.4 and 82.8 mg/kg bw per day during lactation, respectively. During and after exposure, the rats were examined for clinical observations, signs of autonomic dysfunction, abnormal postures, abnormal movements or abnormal behaviour patterns, and unusual appearance daily, beginning on day 0 of gestation at approximately the same time each day. The dams were evaluated for duration of gestation, litter size, live litter size and pup viability at birth. Maternal behaviour of the dams was evaluated on postnatal days 1, 5, 8, 14 and 22. Body weights were recorded daily during the exposure period. Feed consumption values were recorded on day 0 of gestation and daily during the exposure period. Pups were observed for viability at birth, and at least twice daily during the periods before and after weaning. Clinical observations were recorded daily during the period before weaning and weekly during the period after weaning.

On postnatal day 5, litters were reduced to ten pups each. On postnatal day 12, 20 appropriately sized litters per exposure group were selected for continued examination on study. Observations conducted in each of four subsets were as follows: postnatal day 12 brain weights, morphometric measurements and neurohistology examinations of 10 pups of each sex in each of the control group and the group at the highest dose (subset 1), passive avoidance and watermaze testing (subset 2), motor activity and auditory startle habituation testing (subset 3), adult brain weights, morphometric measurements and neurohistology examinations of 10 rats of each sex in the control group and and the group at highest dose (subset 4). One pup per sex was used to standardize litter size to eight pups per litter on postnatal days 12 to 22 and was assigned to subset 5. Rats in subset 4 were observed for signs of autonomic dysfunction, abnormal postures, abnormal movements or abnormal behaviour patterns and unusual appearance by an individual unaware of each exposure group weekly during the period after weaning. Rats assigned to subsets 2 and 3 were examined for gross signs of toxicity when they were weighed or removed from their cages for behavioural testing. Body weights were recorded on postnatal days 1, 5, 8, 12, 14, 18 and 22, weekly during the period after weaning and at sacrifice. Feed consumption values were recorded weekly after weaning beginning on postnatal

day 30. Female rats were examined for the age of vaginal patency beginning on postnatal day 28, and male rats were evaluated for the age of preputial separation beginning on postnatal day 39. The F_1 generation pups/rats selected for continued observation were sacrificed after completion of all after weaning behavioural evaluations. All pups/rats were necropsied; gross lesions were retained.

In the F_0 animals, there were no deaths related to administration of the test substance, and no clinical observations related to the test substance occurred during the study. Maternal body-weight gains during the gestation period were significantly reduced at 300 and 500 ppm on days 0 to 6 of gestation. Average body weights were reduced or significantly reduced at 300 and 500 ppm on days 2 to 16 of gestation (Table 40). Maternal body-weight gains during lactation were significantly reduced at 500 ppm on postnatal days 1 to 4. Average body weights during lactation were significantly reduced at 500 ppm on postnatal days 3 to 8, 11, 13, 17, 21 and 22, and at 300 ppm on postnatal days 13 and 22. Body-weight gains and body weights during gestation and lactation were unaffected by dietary exposure at 50 ppm. A significant reduction in body weight at 50 ppm on postnatal day 22 was not considered to be test substance related because it was a single occurrence. Absolute (g/day) and relative (g/kg per day) feed consumption during gestation were significantly reduced at 300 and 500 ppm for the entire gestation period (calculated as days 0 to 21 of gestation) and on days 0 to 6 of gestation. Absolute feed consumption was also significantly reduced at 500 ppm on days 6 to 9, 9 to 12 and 12 to 15 of gestation. During lactation, absolute feed consumption was significantly reduced at 500 ppm on postnatal days 4 to 7, while relative feed consumption was comparable among the groups.

In the F_1 animals, there were no deaths related to administration of the test substance, and no clinical observations related to the test substance occurred during the study. Body weights in males were significantly reduced at 300 and 500 ppm from postnatal days 8 to 65 and at sacrifice, while body-weight gains at 300 and 500 ppm were significantly reduced at most intervals during the postnatal period. In females, body weights were reduced or significantly reduced at 300 and 500 ppm from postnatal days 8 to 65, while body-weight gains at 300 and 500 ppm were significantly reduced on postnatal days 5 to 8, 8 to 12, 12 to 14 and 18 to 22 (Table 40). There was no effect on male and female body-weight gains and body weights during the postnatal period at 50 ppm.

Absolute (g/day) feed consumption values for males at 300 and 500 ppm were significantly reduced for the entire postnatal period (calculated as postnatal days 30 to 65) and at most intervals during the postnatal period. However, because of the lower body weights at 300 and 500 ppm, the relative (g/kg per day) feed consumption values were significantly increased for the entire postnatal period (calculated as postnatal days 30 to 65) and at most intervals during the postnatal period. In females, absolute (g/day) feed consumption was comparable between the groups. However, because of the lower body weights at 300 and 500 ppm, relative (g/kg per day) feed consumption was significantly increased for the entire postnatal period (calculated as postnatal days 30 to 65) and at most intervals during the postnatal period.

The average day of preputial separation was significantly increased at 300 and 500 ppm (postnatal day 48.2 vs 46.7 in the control group). The delay in preputial separation was considered to be interrelated with the reduced body weights in these exposure groups. There was no effect on preputial separation in the F_1 males at 50 ppm. The average day of vaginal patency was significantly increased at 500 ppm (postnatal days 34.7 vs 33.4 in the control group). The delay in vaginal patency was considered to be interrelated with the reduced body weights in this exposure group. There was no effect on vaginal patency in the F_1 females at 50 ppm.

The behavioural observations and evaluations revealed no evidence for treatment-related effects or biologically important differences between the groups.

At necropsy of pups on postnatal day 12, the relative brain weights were significantly increased in both sexes at 500 ppm. However, this effect was caused by the lower body weights at this dose since the absolute brain weights revealed no significant differences between groups. There were no statistically significant differences in absolute and relative brain weights of animals sacrificed at postnatal days 68–79. Histopathological evaluations and morphometric measurements revealed no evidence for a treatment-related effect.

Table 40. Selected findings in a study of developmental neurotoxicity in rats given diets containing thiacloprid

Finding	Dietary concentration (ppm)			
	0	50	300	500
F_0 animals				
Body weight (g):				
Day 0 of gestation	238.5	239.3	238.8	240.5
Day 4 of gestation	257.7	257.8	247.9**	242.7**
Day 21 of of gestation	407.9	408.2	404.0	399.5
Postnatal day 1	293.8	295.4	286.6	286.2
Postnatal day 13	336.4	323.0	318.2*	312.3**
Postnatal day 22	350.7	330.6*	329.1**	324.0**
Body-weight gain (g):				
Days 0–6 of gestation	28.1	28.8	19.5**	12.4**
Days 0–21 of gestation	169.4	169.2	165.7	158.5
Postnatal days 1–4	13.4	12.7	15.5	4.4*
Postnatal days 1–22	57.6	37.6	40.0	38.8
Feed consumption (g/day):				
Days 0–6 of gestation	23.0	23.0	19.3**	16.1**
Days 0–21 of gestation	27.1	27.0	25.4**	23.7**
Postnatal day 4–7	52.3	50.6	50.2	46.6**
No. of dams pregnant/dams with no liveborn pups	24/0	25/1	24/0	25/0
No. of pups delivered, total/mean	351/14.6	349/14.5	340/14.2	338/13.5
No. of dead pups; Postnatal day 1–5	4	5	4	4
% Male pups	48.6	50.8	50.9	52.6
Mean pup weight (g), postnatal days 1/5	6.8/10.3	6.8/10.4	7.0/10.2	7.1/10.0
F_1 animals				
Body weight (g):				
Postnatal day 5, M/F	10.3/10.0	10.6/10.0	10.4/9.8	10.5/9.9
Postnatal day 30, M/F	80.1/75.3	80.0/74.7	71.0**/67.0**	71.4**/67.2**
Postnatal day 65, M/F	361/234	364/228	335**/223**	336**/227
Body-weight gain (g), postnatal day 5–65, M/F	351/224	354/218	325**/213	325**/217
Feed consumption (g/day), postnatal day 30–65, M/F	25.8/19.9	26.1/19.8	24.5**/19.8	24.2**/19.8
Relative feed consumption (g/kg per day), postnatal day 30–65, m/f	114/122	115/123	119**/129	118**/128
Preputial separation / vaginal patency (day)	46.7/33.4	47.3/33.7	48.2**/33.8	48.2**/34.7**
Terminal body weight (g), postnatal day 12, M/F	23.7/22.4	22.5/21.9	21.6*/20.6	20.3**/19.4**
Brain weight (g), postnatal day 12	1.31/1.28	1.28/1.25	1.27/1.26	1.27/1.25
Relative brain weight (%), postnatal day 12	5.64/5.77	5.79/5.78	5.97/6.11	6.31**/6.36**
Terminal body weight (g), postnatal days 68–79; M/F	420/270	422/245	398/254	382/254
Brain weight (g), postnatal days 68–79	2.30/2.12	2.25/2.05	2.27/2.06	2.20/2.00
Relative brain weight (%), postnatal days 68–79	0.56/0.80	0.54/0.84	0.58/0.82	0.58/0.79

From Hoberman (2001)

* $p < 0.05$; ** $p < 0.01$.

F, females; M, males.

Thiacloprid did not cause any specific neurobehavioural or neuropathological effects in the offspring when administered to the dams during gestation and lactation at dietary concentrations of up to 500 ppm. Non-specific signs of general toxicity, including decreased body-weight gains and delayed sexual maturation, were observed in the offspring at dietary concentrations of 300 ppm and higher.

The NOAEL for maternal toxicity was 50 ppm, equal to 4.4 mg/kg bw per day, on the basis of reduced body weights and feed consumption at 300 ppm and higher. The NOAEL for toxicity to offspring was 50 ppm on the basis of reduced body weights in both sexes and delayed sexual maturation (preputial separation) in males at 300 ppm and higher, and delayed sexual maturation (vaginal patency) in females at 500 ppm (Hoberman, 2001).

(b) Studies on metabolites

(i) Thiacloprid-amide (KKO 2254)

In a study of acute oral toxicity, five male and five female non-fasted Wistar (Hsd/Cpb:WU) rats were given KKO 2254 (purity, 98.8%) as a single dose at 2000 mg/kg bw in demineralized water with 2% v/v Cremophor by gavage. In addition, five males were dosed with 500 mg/kg bw. The study complied with test guideline OECD 401. A single female died in the group at the highest dose. Clinical signs of toxicity were observed in both sexes and included piloerection, decreased motility, spastic and uncoordinated gait, tachypnea, laboured breathing, narrowed palpebral fissure, poor reflexes and spontaneous vocalization. These overt signs were evident between 3–6 h after treatment and lasted for up to 2 days in males and 3 days in females. No gross treatment-related findings were detected at necropsy. The LD_{50} value for the test material was greater than 2000 mg/kg bw for both sexes (Kroetlinger, 1995c).

The mutagenic potential of KKO 2254 (purity, 98.8%; dissolved in DMSO) was investigated in a test for reverse mutation in *Salmonella typhimurium* (TA1535, TA100, TA1537, TA98 and TA102). The study complied with test guideline OECD 471. Six concentrations of up to 5000 µg/plate were used in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). The initial test was performed in triplicate using the plate incorporation procedure, and the results were confirmed in an independent assay using the preincubation (20 min) method. No biologically or statistically significant increase in the number of revertant colonies was seen in any strain at any concentration. No bacteriotoxicity was observed. Appropriate positive controls (sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene) produced significant increases in revertant colonies (Herbold, 1995d).

(ii) Thiacloprid-sulfonic acid Na-salt (WAK 6999)

In a study of acute oral toxicity, five male and five female non-fasted Wistar (Hsd/Cpb:WU) rats were administered WAK 6999 (purity, 95.7%) as a single dose at 2000 mg/kg bw in demineralized water with 2% v/v Cremophor by gavage. The study complied with test guideline OECD 401. No deaths occurred during the study. Clinical signs were observed 4 h after treatment and included diarrhoea and lack of faeces. All overt signs had resolved within 2 days of treatment. No gross treatment-related findings were detected at necropsy. The LD_{50} value for the test material was greater than 2000 mg/kg bw for both sexes (Kroetlinger, 1996d).

The mutagenic potential of WAK 6999 (purity, 95.7%; dissolved in deionized water) was investigated in a test for reverse mutation in *Salmonella typhimurium* (TA1535, TA100, TA1537, TA98 and TA102). The study complied with test guideline OECD 471. Six concentrations of up to 5000 µg/plate were used in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). The initial test was performed in triplicate using the

plate incorporation procedure, and the results were confirmed in an independent assay using the preincubation (20 min) method. No biologically or statistically significant increase in the number of revertant colonies was seen in any strain at any concentration. No bacteriotoxicity was observed. Appropriate positive controls (sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene) produced significant increases in revertant colonies (Herbold, 1995e).

The mutagenic potential of thiacloprid-sulfonic acid Na-salt (purity, 95.1–95.2%; dissolved in deionized water) was tested in a test for gene mutation at the *Hprt* locus in Chinese hamster V79 cells in vitro. The study complied with test guideline OECD 476. Six concentrations of up to 3200 µg/ml were used in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). Under both activation conditions, no cytotoxic effects were induced. No precipitation of thiacloprid-sulfonic acid Na-salt in the medium was observed. However, thiacloprid-sulfonic acid Na-salt was tested up to its limits of solubility under culture conditions. Thiacloprid-sulfonic acid Na-salt induced no biologically relevant increases in mutant frequencies. The positive controls ethyl methanesulfonate and dimethylbenzanthracene had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant frequencies when compared with the corresponding negative controls and thus demonstrated the sensitivity of the test system and the activity of the S9 mix used (Herbold, 2003a).

The clastogenic potential of thiacloprid-sulfonic acid Na-salt (purity, 95.2%; dissolved in deionized water) was tested in an in vitro mammalian chromosome aberration test using Chinese Hamster V79 cells. The study complied with test guideline OECD 473. Cells were exposed for 4 h to thiacloprid-sulfonic acid Na-salt at a concentration of 0, 800, 1600 and 3200 µg/ml in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). Duplicate cultures were used at each concentration. Cells were harvested at 18 h (all concentrations) and at 30 h (0 and 3200 µg/ml). An additional experiment was performed in the absence of metabolic activation using continuous treatment for 18 h, harvesting at the same time, and thiacloprid-sulfonic acid Na-salt at a concentration of 0, 800, 1600 or 3200 µg/ml. From each of two parallel cultures 100 metaphases were examined for chromosome aberrations. In the absence of metabolic activation, cytotoxic effects were observed at 3200 ug/ml after 18 h of treatment. In all other groups treated with thiacloprid-sulfonic acid Na-salt, no cytotoxic effects were observed. None of the cultures treated with thiacloprid-sulfonic acid Na-salt in the absence and in the presence of metabolic activation showed biologically relevant increased numbers of aberrant metaphases. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used (Herbold, 2003b).

(iii) Thiacloprid-sulfonic acid amide

In a study of acute oral toxicity, two groups of three female non-fasted Wistar (Hsd/Cpb: WU) rats were given thiacloprid-sulfonic acid amide (purity, 96.6%) as a single dose at 2000 mg/kg bw in demineralized water by gavage. The study complied with test guideline OECD 423. The dose of 2000 mg/kg bw was tolerated without mortalities, effects on weight development and gross pathology. Clinical signs (decreased motility, decreased reactivity, spastic gait, laboured breathing, uncoordinated gait, lateral position, rolling over and closed eyelids) were evident at 1 h after treatment and lasted for up to 2 days. According to OECD guideline 423, the LD_{50} cut-off of thiacloprid-sulfonic acid amide is 5000 mg/kg bw and higher (Renhof, 2003).

The mutagenic potential of thiacloprid-sulfonic acid amide (purity, 96.6%; dissolved in deionized water) was investigated in a test for reverse mutation in *Salmonella typhimurium*

(TA1535, TA100, TA1537, TA98 and TA102). The study complied with test guideline OECD 471. Six concentrations of up to 5000 µg/plate were used in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). The initial test was performed in triplicate using the plate incorporation procedure, and the results were confirmed in an independent assay using the preincubation (20 min) method. Doses up to and including 158 µg per plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed. At higher doses, the substance had only a weak, strain-specific bacteriotoxic effect. Oing to the weakness of this effect, this range could nevertheless be used for assessment purposes. Precipitation was observed at 5000 µg per plate. Evidence of mutagenic activity of thiacloprid-sulfonic acid amide was not seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls (sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene) had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant colonies compared to the corresponding negative controls (Herbold, 2003c).

The mutagenic potential of thiacloprid-sulfonic acid amide (purity, 96.6%; dissolved in deionized water) was tested in a test for gene mutation at the *Hprt* locus in Chinese hamster V79 cells in vitro. The study complied with test guideline OECD 476. Six concentrations of up to 4000 µg/ml were used in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). In the presence and absence of metabolic activation, thiacloprid-sulfonic acid amide induced no relevant decreases in survival to treatment or decreases in relative population growth. Precipitation of thiacloprid-sulfonic acid amide in the culture medium was also not observed. However, thiacloprid-sulfonic acid amide was tested up to its limits of solubility and up to and greater than 10 mmol/l, the requested limit concentration. In the presence and absence of metabolic activation, there was no biologically relevant increase in mutant frequency above that of the vehicle controls. Ethyl methanesulfonate and dimethylbenzanthracene induced clear mutagenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix (Herbold, 2003d).

The clastogenic potential of thiacloprid-sulfonic acid amide (purity, 96.6%; dissolved in deionized water) was investigated in a test for mammalian chromosome aberration in Chinese Hamster V79 cells in vitro. The study complied with test guideline OECD 473. Cells were exposed in the absence and in the presence of S9 mix for 4 h to thiacloprid-sulfonic acid amide at a concentration of 250, 500, 1000, 2000 or 4000 µg/ml. Cultures at all concentrations were harvested 18 h after the beginning of the treatment. In addition, cells treated at 1000, 2000 and 4000 µg/ml were harvested 30 h after the beginning of the treatment. In the absence of metabolic activation, an additional experiment was performed using continuous treatment for 18 h, harvesting at the same time, and thiacloprid-sulfonic acid amide concentrations of 1000, 2000 and 4000 µg/ml. On the basis of their cytotoxicity, which was additionally determined 8 h after the beginning of the treatment, concentrations were selected for reading of metaphases. In the presence and absence of metabolic activation, no cytotoxic effects were observed. Precipitation in the medium did not occur. Therefore, for all treatment conditions, thiacloprid-sulfonic acid amide concentrations of 1000, 2000 and 4000 µg/ml were chosen for reading. The highest concentration of 4000 µg/ml exceeded the requested maximum concentration of 10 mmol/l. None of the cultures treated with thiacloprid-sulfonic acid amide in the absence and in the presence of metabolic activation showed biologically relevant or statistically significant increased numbers of aberrant metaphases. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used (Herbold, 2003e).

(iv) Comparative study on liver effects in rats

In a short-term study on liver effects, groups of five female Wistar (HsdCpb:WU) rats received diets containing thiacloprid or the metabolites thiacloprid-sulfonic acid amide and thiacloprid-sulfonic acid Na-salt at a concentration of 1000 ppm for 7 days. As controls, five female rats were fed the standard diet. The test compounds were homogeneously distributed and chemically stable for at least 8 days in the diet, and their concentrations in the diet agreed with the target values within defined limits. The daily intakes were 113.08, 125.27 and 115.67 mg/kg bw per day for thiacloprid, thiacloprid-sulfonic acid amide and thiacloprid-sulfonic acid Na-salt, respectively.

No clinical signs were observed, and mortality was not increased in any dose group. The feed consumption per animal and per kg body weight was decreased at all doses. Body-weight development was not influenced by the treatment with the metabolites thiacloprid-sulfonic acid amide and thiacloprid-sulfonic acid Na-salt. Treatment with thiacloprid resulted in a decrease of body-weight development. After 7 days of treatment in this group, the difference to the controls in body-weight gain was –37%.

Table 41. Selected findings in a comparative study of liver effects in rats given diets containing thiacloprid or its metabolites at a concentration of 1000 ppm for 7 days

	Diet			
	Control	Thiacloprid	Thiacloprid-sulfonic acid amide	Thiacloprid-sulfonic acid Na-salt
Body weight (g), day 8	143	138	146	149
Body-weight gain (g)	22	14**	26	28
Liver weight (g)	6.809	8.478**	7.018	7.316
Relative liver weight (%)	4.772	6.145**	4.820	4.911
ECOD (nmol/g per min)	4.0	20.9**	4.2	3.7
EROD (nmol/g per min)	0.44	0.50	0.50	0.44
ALD (nmol/g per min)	31.0	92.7**	32.7	29.5
EH (nmol/g per min)	322	1478**	437	445
GST (µmol/g per min)	101	247**	109	101
UDP-GT (nmol/g per min)	892	2532**	970	924
Aromatase (pmol/g per min)	13.14	25.33**	14.21	11.53

From Kroetlinger et al., (2003)

** $p < 0.01$.

ALD, aldrin epoxidase; ECOD, 7-ethoxycoumarin deethylase; EH, epoxide hydrolase; EROD, 7-ethoxyresorufin deethylase; GST, glutathione-S-transferase; UDP-GT, uridine diphosphate glucuronosyl transferase.

A strong induction of most of the enzyme parameters (phase I and phase II) measured in the liver tissue was detected after administration of thiacloprid at 1000 ppm (Table 41). 7-Ethoxycoumarin deethylase was the most sensitive parameter. The metabolites thiacloprid-sulfonic acid amide and thiacloprid-sulfonic acid Na-salt revealed no induction of the investigated liver enzymes.

Liver aromatase activity measured as the formation of tritiated water was readily detectable in control rats. Treatment with thiacloprid at 1000 ppm approximately doubled aromatase activity in female rat liver. This finding confirmed previous results showing strong induction of liver enzymes including liver aromatase by thiacloprid. In contrast, metabolites of thiacloprid, thiacloprid-sulfonic

acid amide at 1000 ppm and thiacloprid-sulfonic acid Na-salt at 1000 ppm, did not induce liver aromatase activity in female rats (Kroetlinger et al., 2003).

(c) Mechanistic studies on thyroid effects

In a special short-term study of toxicity conducted to investigate the mechanism of thyroidal changes, groups of 10 male and 10 female Wistar (Hsd Cpb:WU) rats were given diets containing thiacloprid (purity, 96.8%) at a concentration of 0, 25, 100, 400 or 1600 ppm for 3 weeks. The mean test substance intakes were equal to 0, 2.6, 9.0, 36.9 and 145.1 mg/kg bw per day in males and 0, 3.1, 12.3, 44.6 and 190.8 mg/kg bw per day in females. The study complied with test guideline OECD 407. The experimental animals were inspected at least once a day. Any clinical signs and abnormalities were recorded. Feed intake and body weight of each animal was deternined weekly.

T3, T4 (total), TBC and TSH levels and protein content were determined from heparinized plasma. The blood for these tests was withdrawn from animals under deep diethylether anesthesia from the retroorbital plexus (days –2 to 14) or by cardiac puncture (days 21 and 22) at predetermined times. UDP-GT was determined from liver samples taken at the time of necropsy. Animals were necropsied after exsanguination under deep ether anesthesia. Liver, thyroid, uterus and pinnae were preserved for microscopic examiation. Liver, uterus and the left thyroidal lobe were weighed at necropsy.

At 1600 ppm, there was a decrement in body-weight development in males (transient) and in females. At 1600 ppm, body-weight gains were significantly reduced by 14.9% in males and by 43.5% in females.

Determination of the thyroidal hormones T3 and T4 after exposure at 1600 ppm resulted in a significant reduction in T3 concentrations on day 2 in male and female rats, and a statistically significant reduction in total T4 concentrations on days 2, 7 and 21/22 in male rats and on day 2 in female rats. Also at 1600 pm, there was a biologically relevant increase in the concentration of TSH in males on day 14 and in females on days 7 and 22 (Table 42). There was considerable variation within the observations made in the control groups at different times. This was not unexpected as hormone determinations usually result in wide intra-individual variation. In order to interpret the hormone data more intelligently with regard to their biological relevance, an analysis of covariance has been made of the T3, T4 and TSH data. This comparison was based on the measurements before treatment (day –2).

The comparison of the relative changes in thyroid parameters (%) indicated that there were significant changes only at 1600 ppm. There were reductions in T3 and T4 on day 2 of exposure. In male rats, T4 values were depressed persistently throughout the study and in female rats on days 7 and 22. The concentrations of TSH were increased in rats starting from day 7. In females, this increase in TSH was only seen at 1600 ppm. Because of wide intra-individual variation, this effect was statistically significant in female rats only on days 7 and 22. In male rats, the most pronounced increase in TSH concentrations was invariably seen at 1600 ppm. There were also some increases at 100 ppm (days 14 and 21) and at 400 ppm (days 7–21), which were above the range of the increases seen at less than 100 ppm. However, the effects observed at 100 and 400 ppm on days 7 and 14 were not dose-related, and, thus, the effects seen at 100 ppm were considered to be spurious results caused by wide intra-individual variation, while the effects seen at 400 ppm, which were consistently seen on days 7–21 were considered to represent an incipient response of the pituitary, which was more markedly expressed at 1600 ppm.

The activity of UDP-GT, the enzyme catalysing the glucuronidation of T4, was significantly induced in both sexes at greater than 400 ppm at the time of necropsy. There was no evidence of enzyme induction at less than 100 ppm. At the end of the exposure period, there was a slight increase in protein concentrations in male and female rats at 1600 ppm. There was an increase in absolute

and relative liver weight in animals at the highest dose. The apparently statistically significant reductions of thyroid weights in females (absolute at > 400 ppm, relative at 400 ppm) were not regarded as treatment-related, as there was no dose–response relationship for the relative thyroid weight. Histopathology of the thyroid gland revealed treatment-related hypertrophy of the follicular epithelium in males at 400 ppm and higher and in females at 1600 ppm, with statistical significance in both sexes at 1600 ppm.

Table 42. Selected findings in a special short-term study in rats fed diets containing thiacloprid for 3 weeks

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	25	100	400	1600	0	25	100	400	1600
T3 (nmol/l):										
Day –2	2.24	2.24	2.34	2.37	2.23	2.36	2.26	2.37	2.45	2.33
Day 2	2.08	2.07	2.23	2.26	1.75*	2.19	2.04	2.29	2.25	1.60**
Day 7	1.86	1.88	1.84	1.84	1.79	1.94	1.96	2.13*	2.16*	1.92
Day 14	1.87	1.98	1.92	1.98	1.81	1.95	1.87	1.95	2.17	2.17*
Day 21/22	1.93	1.90	2.03	1.99	2.02	1.81	1.83	1.98**	2.12**	2.15**
T4 (nmol/l):										
Day –2	117	126	121	127	124	113	110	100	118	102–
Day 2	110	110	112	124	84*	98	94	92	115	67*
Day 7	114	120	118	119	93*	102	97	96	107	80
Day 14	126	143	130	139	109	109	121	113	125	105
Day 21/22	95	95	93	94	75**	72	79	87	86*	59
TSH (µg/l):										
Day –2	2.42	3.46*	2.03	2.44	1.91	1.83	1.34	1.65	1.59	1.80
Day 2	3.56	3.90	2.81	2.41	1.29**	2.38	1.67	1.94	2.20	1.99
Day 7	2.81	3.94	3.33	5.40	4.33	1.67	1.39	1.64	2.09	3.87**
Day 14	3.54	4.67	6.61*	6.06*	10.68*	2.97	1.91	2.13	2.00	3.47
Day 21/22	3.26	5.96	5.62	5.36	6.77	2.10	1.76	1.61	1.83	5.54**
UDP-GT (nmol/g per min)	760	658	813	1507**	2676**	554	483	636	909**	2332**
Plasma protein (g/l)	65.7	64.5	64.3	64.1	68.8**	66.0	66.8	67.1	65.9	70.0**
Body weight (g):										
Day 7	209	209	211	214	187**	154	151	151	150	134**
Terminal	269	271	278	289	254	184	175	185	182	162**
Liver weight (g)	11.22	10.58	11.15	12.82*	14.83**	7.53	6.89	7.56	7.79	9.25**
Relative liver weight (%)	4.158	3.900	4.002	4.43	5.85**	4.09	3.92	4.09	4.29	5.70**
Thyroid weight (mg)	10	8	9	11	10	7	5	5	4*	4*
Relative thyroid weight (mg/100g)	4	3	3	4	4	4	3	3	2*	3
Follicular cell hypertrophy	1	1	3	5	8**	0	0	1	2	5*
Grade 1	0	1	3	3	3	0	0	1	2	3
Grade 2	1	0	0	2	5	0	0	0	0	2

From Andrews (2000)

T3, triiodothyronine; T4, thyroxin; TSH, thyrid-stimulating hormone; UDP-GT, uridine diphosphate glucuronosyl transferase

* $p < 0.05$,

** $p < 0.01$

The NOAEL was 100 ppm, equal to 9.0 and 12.3 mg/kg bw per day in males and females, respectively, on the basis of thyroid effects and induction of UDP-GT at 400 ppm and higher (Andrews, 2000).

Studies (that did not comply with GLP or guidelines) were carried out to investigate the possibility that thiacloprid (purity, 98.6%) or its metabolites could exert a direct effect on thyroid peroxidase (TPO) in vitro. Interactions of thiacloprid at 435 or 870 µmol/l with TPO-catalysed reactions were evaluated using a partially purified fraction of hog thyroid glands as an enzyme source. TPO-catalysed guaiacol oxidation and iodine formation were used as measures for peroxidase activity. Plasma extracts from rats treated with thiacloprid at 2000 ppm for 14 days were also screened for an inhibitory effect on TPO-catalysed iodine formation. The results show that thiacloprid neither inhibited the TPO-catalysed guaiacol oxidation nor the TPO-catalysed iodine formation from iodide at a concentration of 435 µmol/l (IC_{50} values > 870 µmol/l). The plasma extracts also had no inhibitory effect on the TPO-catalysed iodine formation. Therefore, it was concluded that thiacloprid and its metabolites had no direct inhibitory effect on TPO (Freyberger, 1994).

(d) Mechanistic studies on aromatase induction

In a mechanistic study, groups of 30 female $B6C3F_1$ mice were given diets containing thiacloprid (purity, 97–97.2%) at a concentration of 0, 30, 250 or 2500 ppm for 13 weeks. Interim kills (15 animals per dose) were made at approximately 4 weeks. An additional group of 30 female mice was treated with thiacloprid at 2500 ppm and also received drinking-water containing 0.005% mecamylamine, a nicotine-mimicking agent. This satellite group of animals was used to investigate the possibility that the effects on the adrenal X-zone were centrally mediated by nicotinergic transmission. The study was performed in compliance with test guideline OECD 407. Clinical signs of toxicity, body-weight changes, feed consumption and water intake were recorded at suitable time-points. Vaginal smears were taken from each female before mating, examined microscopically and classified as diestrus, proestrus or estrus. Blood samples were collected by cardiac puncture at the interim and final necropsy. However, hormone determinations were only performed on the samples obtained at the terminal necropsy. All the animals were necropsied in the diestrus phase and examined macroscopically. The following organs were removed and weighed: brain, heart, kidneys, liver, spleen, thymus, adrenals, ovaries, uterus and pituitary gland. Liver samples of six mice per group were used for aromatase determinations. An extensive list of organs and tissues were examined microscopically. The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes were equal to 0, 6, 18, 139, 1101 and 1244 mg/kg bw per day in females at of 0, 10, 30, 250, 2500 and 2500 ppm (satellite group), respectively.

No treatment-related deaths occurred during the study. Decreased motility and reactivity were observed at doses of 250 ppm or higher and reduced feed consumption at 2500 ppm (22 and 12%). There were no effects on water intake or body weight. No macroscopic findings were observed at necropsy. The mean liver weights (absolute and relative) were significantly increased in both groups at 2500 ppm (Table 43). There was a small increase at 4 weeks in mean relative liver weight (8%) at 250 ppm, which was not seen at termination.

A dose-related increase in vacuolation of the X-zone of the inner adrenal cortex was found at doses of 250 ppm and higher at 4 and 13 weeks. At 2500 ppm, the marked vacuolation resulted in enlargement of the X-zone at 13 weeks.

The aromatase activity of liver tissue was significantly induced at doses of 250 ppm and higher after 13 weeks.

Progesterone concentrations were slightly increased at 2500 ppm. Estradiol concentrations were slightly reduced at 250 and 2500 ppm, but the estradiol/progesterone ratio was only reduced

at 2500 ppm. The administration of mecamylamine did not abolish the effect of thiacloprid at 2500 ppm on the estradiol/progesterone ratio. This demonstrates that the thiacloprid-mediated effects on the adrenal X-zone and hormone changes were not caused by central nervous events, which can be counteracted by the nicotine-like agent mecamylamine.

Table 43. Selected findings in a study of aromatase induction in mice fed diets containing thiacloprid for 13 weeks

Finding	Dietary concentration (ppm)					
	0	10	30	250	2500	2500[a]
Interim sacrifice						
Body weight (g)	22	22	21	21	21	21++
Liver weight (g)	1.10	1.09	1.06	1.17	1.43**	1.31**
Relative liver weight (%)	5.04	5.00	4.97	5.44*	6.67**	6.31**
Terminal sacrifice						
Body weight (g)	24	26**	24	24	25	24
Liver weight (g)	1.20	1.20	1.14	1.17	1.51**	1.41**
Relative liver weight (%)	4.97	4.69	4.69	4.81	6.14**	5.94**
Estradiol (pg/ml), week 13	35.2	37.8	37.5	32.3	31.5	28.3
Progesterone (ng/ml), week 13	1.48	1.57	1.43	1.45	2.02	2.10
Estradiol/progesterone ratio, week 13	26.1	28.8	28.0	32.0	17.4	15.5
Aromatase (pmol/g per min)	11.9	–	14.5	19.6**	56.2**	–
Adrenal X zone vacuolation, No. examined	15	14	15	15	15	15
Grade 1	9	9	10	1	0	1
Grade 2	3	5	4	7	1	1
Grade 3	0	0	0	7	2	5
Grade 4	0	0	0	0	10	7
Grade 5	0	0	0	0	2	1
Total	12	14	14	15	15	15
Average grade	1.0	1.3	1.2	2.4	3.9	3.4

From Andrews et al. (1998)

[a] Plus drinking-water containing mecamylamine.

* $p < 0.05$; ** $p < 0.01$.

Continuous stimulation of the ovarian tissue could explain the increased incidence of ovarian luteomas observed in the study of carcinogenicity. The ovarian and adrenal effects in mice were interpreted as secondary to the hormonal imbalance induced by the liver enzyme/aromatase induction.

The NOAEL for female mice was 30 ppm, equal to 18 mg/kg bw per day, on the basis of clinical signs, enzyme induction and adrenal effects at 250 ppm (Andrews et al., 1998).

Two serial studies had been conducted to investigate the effect of administration of dietary thiacloprid (purity, 96.2–97.2%) on the activity of aromatase in male and female rats and to provide additional toxicokinetic data. The studies complied with test guideline OECD 407. In the first study, groups of 15 male and 15 female Wistar (Hsd Cpb:WU) rats were given diets containing thiacloprid at a concentration of 0, 100, or 1000 ppm for 4 weeks. In the second study, groups of 10 female Wistar (Hsd Cpb:WU) rats were given diets containing thiacloprid at a concentration of 0, 200 or 500 ppm for 4 weeks. Clinical observations, body weight and feed intakes were recorded at appropriate time-points.

Vaginal smears were taken, examined microscopically and classified as diestrus, proestrus or estrus. Blood samples were taken from the orbital plexus on days 1, 8, 15, 22 and 28 (first study). Female rats were necropsied in the diestrous phase. Male rats were necropsied at the end of treatment. Selected organs (brain, adrenals, ovaries and liver) were removed and weighed. The liver (first and second studies) and ovaries (first study) were used for enzyme determinations. At necropsy, blood was taken from female rats (first study) in the diestrous stage of the estrous cycle for hormone determinations. These hormone determinations are stated to be flawed because of systematic errors, therefore they were not reported.

The concentrations in diet were confirmed by analysis. Homogeneity and stability have been shown to be acceptable in previous studies. The mean daily intakes in males were equal to 0, 6.7 and 66.7 mg/kg bw per day at 0, 100 and 1000 ppm, respectively. In females, the mean daily intakes were equal to 0, 6.6, 20.4, 47.5 and 60.4 mg/kg bw per day at 0, 100, 200, 500 and 1000 ppm, respectively.

No deaths occurred during the study. Lightly coloured faeces were observed in some animals at 1000 ppm. Significant reductions in mean body-weight gain were observed in males (–27%) and females (–80%) at 1000 ppm and in females at 500 ppm (–39%). Feed intake was not affected by treatment.

The plasma concentrations of thiacloprid in males at 1000 ppm had reached a plateau at approximately 60–64 nmol/ml for days 1–8, but a slight decrease was evident from day 15 onwards. In females at 1000 ppm, the mean plasma concentration increased up to day 7 and reached a plateau at around 80–100 nmol/ml. A 10-times increase in dose resulted in an 8-times increase in the mean plasma concentration in males and a 14-times increase in females (Table 44). A decline in the plasma concentrations during the determination period, which would be expected as a consequence of enzyme induction, did not occur. The kinetic data revealed that despite enzyme induction, mean plasma concentratios of thiacloprid remained relatively stable in female rats at 100 ppm and 1000 ppm throughout the study. A slight decrease in mean plasma concentrations was detected in male rats at the highest dose. The higher plasma/dose ratio seen in females at 1000 ppm may indicate saturation of the liver metabolic capacity in females at the highest dose.

Table 44. Selected findings in a study on aromatase induction in rats fed diets containing thiacloprid for 4 weeks

Finding	Dietary concentration (ppm)							
	Males			Females				
	0	100	1000	0/0[a]	100	200[a]	500[a]	1000
Plasma concentration of. thiacloprid (nmol/ml):								
Day 1	—	6.7	59.9	—	4.4	—	—	51.6
Day 8	—	7.1	64.3	—	6.8	—	—	96.8
Day 15	—	7.3	52.1	—	6.1	—	—	89.6
Day 22	—	7.3	48.7	—	6.1	—	—	80.4
Day 28	—	5.8	52.3	—	5.5	—	—	84.0
Body weight (g), day 28	341	323	314**	230/201	234	188	183*	217**
Liver weight (g), day 28	12.56	12.78	15.03**	8.36/7.69	8.52	7.24	7.69	9.25*
Relative liver weight (%), day 28	3.74	3.90	4.76**	3.59/3.83	3.61	3.85	4.20**	4.21**
Aromatase, liver (pmol/g per min)	—	—	—	10.4/9.1	9.3	16.4**	19.2*	23.0*
Aromatase, ovary (pmol/g per min)	—	—	—	4.2/—	4.3	—	—	4.4

From Andrews et al. (1998)

[a] Second study.

* $p < 0.05$; ** $p < 0.01$.

No macroscopic changes were detected at necropsy. Liver weights were increased at doses of 500 ppm or higher. A dose-related increase in aromatase activity was detected in the liver: by 1.8-fold at 200 ppm, 2.1-fold at 500 ppm and a 2.4-fold at 1000 ppm.

The NOAEL for aromatase induction in the liver was 100 ppm, equal to 6.6 mg/kg bw per day. In the ovaries, which are the main site of steroid production and aromatase activity, no induction of aromatase by thiacloprid was evident (Andrews et al., 1998).

(e) Mechanistic studies on reproductive effects (dystocia, stillbirths)

In a supplementary one-generation study of reproduction, groups of 15 male and 30 female Sprague-Dawley rats were given diets containing thiacloprid at a concentration of 0, 25, 300 or 1000 ppm. The animals were exposed to the test material throughout the entire study, which included a 10-week period before mating. The purpose of this study was to determine the reproducibility of dystocia and increase in stillbirths observed in the two-generation study of reproduction. Clinical observations, body weight, feed intake and the routine reproductive parameters were recorded at appropriate time-points. In addition, the time between the initiation of labour and the first birth, and the times between the births of pups was evaluated in some dams. Gross necropsy evaluations were performed on all adult females. Selected organs were removed and retained in fixative. The liver and thyroid were weighed. No histopathological examinations were performed. The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes at 0, 25, 300 and 1000 ppm were equal to 0, 2, 20 and 69 mg/kg bw per day in males and 0, 2 (2, 2), 23 (20, 35) and 75 (68, 119) mg/kg bw per day in females (gestation and lactation intakes in parentheses), respectively.

There were six unscheduled deaths in females at 1000 ppm; the cause of death was undetermined. Four of the six females were found dead on days 22–24 of gestation, with two of these animals having started to deliver at the time of death. One female was found dead on day 40 (before mating), and one female was sacrificed in a moribund condition on day 134 (sperm-positive with copulatory plug/no implants). Clinical signs of toxicity were observed at 1000 ppm and included paleness, laboured breathing and cold to touch. The mean body weights of females at 1000 ppm were significantly lower during the last 3 weeks of the phase before mating (6%), during gestation (6–12%) and during days 0–4 of lactation (10–13%). No treatment-related effects on feed consumption were noted during the study.

There were no treatment-related effects on time to insemination, mating, duration of gestation or pup sex. The time between the initiation of labour and birth or the time between births was not affected by treatment.

There was a significant and treatment-related decrease in the pup viability index at 1000 ppm (98% and 76% for the control and 1000 ppm, respectively). Also, the mean pup weights were significantly decreased (14%) on day 4 at 1000 ppm. A greater number of pups and litters at the highest dose were considered to be weak. This correlated with the decrease in the viability index (Table 45).

Table 45. Selected findings in a one-generation study of reproduction in rats fed diets containing thiacloprid

Finding	Dietary concentration (ppm)			
	0	25	300	1000
Body weight (g), females, terminal	260.9	264.0	251.5	241.3*
Liver weight (g), females	10.029	10.694	10.604	12.231*
Relative liver weight (%)	3.841	4.041	4.209*	5.056*
Thyroid weight (g), females	0.017	0.016	0.017	0.020*
Relative thyroid weight (%)	0.0064	0.0062	0.0066	0.0085*
No. of animals mated	30	30	30	28

No. of animals delivered	27	25	25	20
No. of animals with implants	27	26	25	20[g]
Mating index	100.0	100.0	100.0	93.3
Fertility index	90	86.7	83.3	89.3
Gestation index	100.0	96.2	100.0	80.0
Duration of gestation (days)	22.3	22.2	22.3	22.3
Total No. of implantations	349	320	313	233
Mean implantations	12.9	12.3	12.5	11.6
Total No. of pups born	337	292	291	198
Total No. of pups found dead / No. of litters	4/3	3/2	9/6	14/6
Mean litter size	12.5	11.7	11.6	9.9
Birth index	92.7	87.3	92.6	85.5
% Male pups at birth	48.6	48.4	44.9	52.6
No. of stillborn pups	13	5	15	15
Live birth index	96.5	98.3	94.9	91.1
No. of weak pups / No. of litters	1/1	0	0	10/4
Viability index	97.6	98.0	95.0	76.4*
Mean weight of viable pups at birth (g)	6.7	6.6	6.8	6.5
Mean weight of viable pups on day 4 (g)	10.3	10.4	10.2	8.9*

From Eigenberg (1998a)

* $p < 0.05$; ** $p < 0.01$.

There were no treatment-related gross findings at necropsy. Absolute and relative liver weights were increased at 1000 ppm (by 22% and 32%, respectively), while the relative liver weights were also increased at 300 ppm (10%). Absolute and relative thyroid weights at 1000 ppm were increased by 18% and 33%, respectively (Table 45).

In the group at the highest dose, two dams that died were considered to be dystocic owing to the delivery of pups followed by a long period of time with no further deliveries. One dam delivered one pup on day 22 of gestation (13:15 h) but delivered no further pups. This dam was found dead on the morning of day 23 of gestation (12 pups found in utero at death). The second dam delivered 10 pups on day 23 of gestation (07:40 h). This dam was found dead on the morning of day 24 of gestation (six pups found in utero at death). A third dam considered to be dystocic was found dead on day 24 of gestation (most dams deliver on day 22 or 23 of gestation) without ever having been observed in labour (nine pups found in utero at death). On day 4 of lactation, reduced viability and body weight were noted in the pups at 1000 ppm. There were significant increases in mean relative liver weight at 300 (9.6%) and 1000 ppm (31.6%). The dystocia and lack of initiation of labour may have been due to the marked toxicity seen at the highest dose (death, clinical signs, body weight effects and increased organ weights).

The NOAEL for systemic toxicity was 300 ppm, equal to 20 mg/kg bw per day, on the basis of clinical signs (including deaths), decreased body weight, and increased liver and thyroid weights at 1000 ppm. The NOAEL for reproductive toxicity was 300 ppm on the basis of dystocia and decreased pup viability at 1000 ppm (Eigenberg, 1998a).

In a study to investigate the cause of dystocia and stillbirths in rats treated with thiacloprid, groups of 30 male and 115 female CD Sprague-Dawley rats were given diets containing thiacloprid (purity, 96.7–97.0%) at a concentration of 0 or 1000 ppm during a 10-week pre-mating period, mating and gestation. During the study, animals were evaluated for the effect of the test compound on body weight, feed consumption, and clinical signs.

The following procedures were performed on the cervix to evaluate changes which occur during pregnancy. The cervix was examined morphologically for decreases in the organization of collagen, concentration of collagen, and increased vascularization. Cervical mass and water content were determined. Cervical extensibility was determined. Collagen organization was evaluated in vivo. For the uterus, the following procedures were performed. Electromyographic activity (EMG) and mechanical activity (intrauterine pressure) were evaluated in vivo. Uterine contraction was evaluated in vitro using oxytocin to stimulate contractions and isoproterenol (an alpha agonist) to inhibit oxytocin-stimulated uterine contractions. Morphological changes (histology and tissue weight) of the uterus were evaluated. Alpha-1 adrenergic receptor binding in the uterus was also investigated as a measure of compound-related dysfunction mediated via effects on tissue innervation.

The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes at 1000 ppm were equal to 62 mg/kg bw per day in males and 73 mg/kg bw per day in females.

There were no treatment-related clinical signs. Four deaths occurred during the study at 1000 ppm. There was no treatment-related effect on male body weights. For females treated with 1000 ppm, significantly lower body weights were observed beginning on day 14 of the phase before mating.

Cervical extensibility: in one investigation, pregnant rats were sacrificed on gestation days 16 (six per dose), 21 (six per dose) and at term (two treated and four controls). In a second independent investigation, pregnant rats (10 per dose) were sacrificed on day 21 of gestation. The cervices were collected before delivery. All fetuses were counted and weighed. Each cervix was suspended between two hooks in an oxygenated organ bath at 37 °C. One hook was connected to a cervimeter, a special instrument for extensibility determinations. The cervix was stretched in 0.1 mm increments at 1-min intervals. The resulting force was recorded by a computer connected to the cervimeter. The change in slope of extensibility was an indicator of cervical extensibility and a reduction of the slope served as a measure of an increase in extensibility. No statistically significant difference in cervical extensibility between controls and treated animals was observed on days 16 and 21 of gestation, which was also valid for day 22 of gestation, although at that time the group size was lower.

Cervical mass and water content: twenty pregnant rats (10 per dose) were used to evaluate cervical wet and dry weight. The cervix of the animals was removed on day 21 of gestation. The wet weight was determined after cleaning the cervix from surrounding connective tissue and fat. Dry weight was determined after lyophilization. There were no treatment-related effects on the cervical wet and dry weights.

Cervical collagen content: the effect of thiacloprid on cervical collagen was tested from day 13 of gestation until term using light-induced fluorescence measurements. The probe consisted of a fibreoptical probe and a sheath for isolating the optical fibre from the measuring site. This probe was inserted in the vagina with a sapphire window in contact with the surface of the external cervical os for measurement. A total of nine control rats and six treated rats were evaluated. Two measurements per time-point were performed every other day starting on day 13 of gestation. On day 22 of gestation, the measurements were recorded at the time of delivery where possible. The rats that had not delivered and the rats that were in the delivery process at the time of the measurement were separated into two different groups: day 22 non-delivery and day 22 delivery. The results indicated a cervical hardening on days 13, 15 and 17 of gestation for treated rats when compared with control animals. No differences were obvious on days 19 and 21 of gestation. However, marginal cervical hardening was noted on day 22 of gestation in the non-delivery and delivery treatment groups. Since this slight hardening was seen in both the treated non-delivery and delivery groups, this marginal effect did not appear to have a negative effect on birth. In addition, the observed mid-gestation changes did not appear to affect the birth process.

Contractile activity of isolated uterine and cervical tissue: in this investigation, the effect of administration of thiacloprid on the contractile activity of isolated uterus and cervical tissue was tested using oxytocin (induces contraction) and isoproterenol (inhibits contractions). Pregnant rats

(four to six rats per dose) were sacrificed on days 16 and 22 of gestation. The uterus and cervix were dissected free of the surrounding tissues and cut into rings of 4 mm width. These rings were mounted on stirrups in organ chambers containing Krebs-Henseleit solution at 37 °C. The lower stirrup was fixed to the bottom of the organ chamber and the other was connected to an isometric force transducer. Passive tension was applied to the uterine and cervical rings up to basal tension at a weight of 2 g. After the basal tension was stabilized either oxytocin (10^{-10}–10^{-6}°mol/l) or isoproterenol (10^{-11}–10^{-6}°mol/l) were added incrementally to the organ chamber and the contractility was measured in the control and thiacloprid-treated animals.

The concentration–contraction relationship of oxytocin was similar for both treated and control animals on days 16 and 22 of gestation. Although there was a tendency towards weaker activation of spontaneous rhythmic contractions by oxytocin in rats treated with thiacloprid, this was not considered to be biologically significant. Since the minimal concentration of oxytocin produced more than a 50% increase in rhythmic activity in most of the uterine rings, the EC_{50} value could not be calculated.

The concentration–contraction relationship of isoproterenal was similar for both treated and control animals on days 16 and 22 of gestation. No statistically significant difference in the median inhibitory concentration (IC_{50}) values for isoproterenol-induced reduction of contractile activities in uterine and cervical tissue was detected. A slight decrease in IC_{50} was observed on day 22 of gestation when compared with day 16, but this was not statistically significant. Thus, it was concluded that basal spontaneous rhythmic activity or the inhibition induced by the β-adrenergic agonist isoproterenol was not affected by thiacloprid.

Uterine electromyographic activity and intrauterine pressure: to measure the change in uterine electrical activity and intrauterine pressure in rats from day 18 of gestation until delivery telemetric implants were used. For this purpose electrodes were sutured to the uterine wall, at approximately mid-distance between the ovarian and cervical ends of the uterine horn. Each pair of electrodes consisted of a pick-up electrode and a ground electrode which were approximately 2–3 mm apart. Intrauterine pressure was measured by placing a pressure transducer in the uterine cavity, which was also connected with the telemetric recording system. Uterine electromyographic (EMG) activity (duration, amplitude and number of bursts, power density at different frequencies, integral of electrical activity) and pressure were recorded each day, beginning on day 18 of gestation for 4 h, continuous recordings were collected from day 21 of gestation until delivery. The intrauterine pressure was determined as the strength of contraction multiplied by the time unit.

With regard to electrical burst results, despite some variability, this parameter and the resulting integral of electrical burst and maximum power density values showed no difference between thiacloprid-treated animals and controls through the duration of the pregnancy. In addition, intrauterine pressure was not different between treated and control groups.

Alpha receptors: the uteri from pregnant rats (15 per dose) sacrificed on day 21 of gestation were evaluated for alpha-1 adrenergic receptors. The mean alpha-1 receptor levels for the control and thiacloprid-treated dams were 3.85 and 3.89 pmol/mg protein, respectively. Thus, exposure to thiacloprid did not affect uterine alpha-1 adrenergic receptor levels.

A routine gross necropsy was performed on the 10 animals used for histological evaluation of the cervix and uterus. At necropsy, the following data was recorded for each dam: terminal body weight, ovary, cervical, vaginal and empty uterine weights and the number of fetuses.

No gross treatment-related findings were seen at necropsy. The terminal body weight of females at 1000 ppm was reduced significantly (15%). There was a significant decrease in the number of fetuses per litter in dams at 1000 ppm used for the pathological investigations (7.7 and 12.9 fetuses per litter for the group at 1000 ppm and the control group, respectively). When the number of fetuses per litter from the studies of cervical extensibility and uterine contractility were combined with the number of fetuses per litter used for the pathological investigations, a significant decrease in the number of fetuses per litter was also evident at 1000 ppm (10.2 and 12.3 fetuses per

litter for the group at 1000 ppm and the control group, respectively). Microscopy did not reveal any treatment-related effects on the cervix or uterus.

The treatment-related effects observed at 1000 ppm included an increase in deaths, reduced body weight and reduced litter size. The functional and morphological investigations did not reveal any compound-related effects on the cervix or uterus. Thus, the Meeting concluded that thiacloprid did not have a direct effect on birth functions (Eigenberg, 1998b).

In a study of reproduction in rats conducted to determine whether dystocia could be induced by short-term administration of thiacloprid, groups of 30 pregnant Sprague-Dawley rats were given thiacloprid (purity, 97.0%) at a dose of 0, 35 or 60 mg/kg bw per day by gavage initially on days 18–21 of gestation. The vehicle was aqueous 0.5% (w/v) carboxymethyl cellulose with 0.4% (v/v) Tween 80. Because of severe toxicity and deaths in the groups at 35 and 60 mg/kg bw per day, an additional dose of 17.5 mg/kg bw per day was introduced into the study. This third group of animals was created from the rats at 0, 35 and 60 mg/kg bw per day that had not reached day 18 of gestation (not previously dosed with vehicle or thiacloprid). Dose selection was based on a previous study (Eigenberg, 1998c) in which rats were dosed by gavage at 100 mg/kg bw per day on days 18–19 of gestation and at 50 mg/kg bw per day on day 20 of gestation. This study did not show treatment-related dystocia but did show that thiacloprid at 100 mg/kg bw per day was extremely toxic during the terminal period of pregnancy. Clinical observations, body weight and feed intake were recorded at appropriate time-points. The number of live and stillborn pups was recorded. A gross necropsy was performed on dams sacrificed moribund or found dead on days 18–21 of gestation.

The analysed concentrations of the dosing solutions were at least 95% of the target concentration. Twelve animals were found dead and four animals were sacrificed in a moribund condition during days 20–24 of gestation: 1 out of 27 (sacrificed moribund), 0 out of 9, 7 out of 29 (two sacrificed moribund) and 8 out of 25 (one sacrificed moribund) at 0, 17.5, 35 and 60 mg/kg bw per day, respectively. Clinical signs of toxicity were seen at doses of 35 mg/kg bw/ per day or higher and included hypoactivity, chromorhinorrhoea and clear vaginal discharge.

The mean body weights of the rats in groups at 35 and 60 mg/kg bw per day were significantly lower than those of the control group during the dosing period (14% lower on day 21 of gestation for both groups). There was a significant dose-related reduction in mean body-weight gain at all doses. Significant reductions in feed intake were seen on days 18–21 of gestation at all doses (Table 46).

Table 46. Selected findings in a special study of reproduction in rats given thiacloprid by gavage

Finding	Dose (mg/kg bw per day)			
	0	17.5	35	60
No. of animals	27	9	29	25
No. of dead/sacrificed animals	1	0	7	5
Body weight (g), day 21	385.0	376.8	330.0*	332.4*
Body-weight change (g), days 18–21	41.3	13.3*	–18.3*	–24.9*
Feed consumption (g/kg per day), days 20–21	69.0	34.4*	9.8*	4.3*
No. pregnant	21	9	29	16
No. of litters	21	9	22	11
Mean litter size	12	12	10	12.7
Total No. of pups	257	109	231	128
No. of live births	253	102	192	81
No. of stillborn pups (% of total pups).	4 (1.6)	5[a] (4.6)	28 (12.1)	34 (26.6)
No. cannibalized	0	2	11	13

No. cannibalized	0	2	11	13
Mean No. of viable pups	12	11	9	7
Live birth index (mean)	98.9	94.1	83.0*	71.1*

From Eigenberg (1998d)

[a] One litter had three stillbirths + one partially cannibalized pup and one litter had two stillbirths + one partially cannibalized pup.

* $p < 0.05$; ** $p < 0.01$.

A dose-related increase in the incidence of stillbirths was evident at 35 mg/kg bw per day and higher. When the low group size and cannibalization were taken into consideration, it was possible that a slight increase in stillbirths could have been occurring at 17.5 mg/kg bw per day.

The NOAEL for systemic toxicity was less than 17.5 mg/kg bw per day on the basis of decreased body-weight gain and feed intake at a dose of 17.5 mg/kg bw per day and higher. Dystocia was not observed, but there was an increase in the incidence of stillbirths at doses of 35 mg/kg bw per day or higher. Thus, the NOAEL for reproductive effects was 17.5 mg/kg bw per day (Eigenberg, 1998d).

In a modified one-generation study designed for examination of the increased incidence of dystocia and stillbirths observed in reproductive bioassays, male and female Sprague-Dawley rats were given diets containing thiacloprid (purity, 97%) at a concentration of 0 or 800 ppm for 10 weeks for males (phase before mating) and up to 14 weeks for females (before mating, gestation, lactation). All animals were aged 6–8 weeks when exposure to thiacloprid was initiated. The concentration, stability and homogeneity of the test material in the diet were acceptable. At 800 ppm, the mean daily intakes were equivalent to 54.0 mg/kg bw in males and 60.4 mg/kg bw in females. Groups of 10–16 female rats per dose were sacrificed after: (i) a 9 ± 1 week period before mating; (ii) a before-mating + mating/pregnancy + gestation phase concluding with sacrifice on day 18 or 21 of gestation (day 0 = sperm-positive); and (iii) a postnatal phase with sacrifice on postnatal day 2 (day 0 = delivery). The estrous cycle was monitored by daily vaginal cytology; only rats that showed at least two consecutive 4-day estrous cycles were used in this study. At the sacrifice times, rats were asphyxiated in a CO_2 chamber and terminated by exsanguination.

Plasma for determination of circulating oxytocin levels was collected on day 21 of gestation; serum was collected at all other time-points. The standard clinical chemical parameters were assayed. Hepatic microsomal enzyme assays were conducted in perfused liver on cytochrome P450, *N*-demethylase and *p*-nitroanisole *O*-demethylase. Reduced glutathione was measured in liver and in uterus. Uterine and cervical prostaglandin E_2 and $F_{2\alpha}$ content were measured. Blood hormone determinations were made for luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, progesterone, corticosterone, T4, T3, TSH, oxytocin and prolactin after the phase before mating (week 9), on days 18 and 21 of gestation, and at postnatal day 2. In addition, estrogen and progesterone receptor levels were determined in the cytosolic and nuclear fractions of the uterus. At each sacrifice, liver, uterus, ovaries, mammary gland, adrenals, pituitary and cervices were weighed, and tissue specimens collected for biochemical and/or histopathological analysis. The hypothalamus was collected but not weighed. Histopathological examinations of the liver, adrenals, uterus, ovaries, mammary gland, cervix, pituitary and hypothalamus-related tissue were carried out.

One female was found dead on day 50 and two pregnant females were sacrificed owing to prolonged or incomplete parturition. One of the pregnant females showed only slight indications that parturition had been initiated and did not deliver. The second female successfully delivered several pups but did not complete the delivery process (two live and two dead pups were found in the uterus at necropsy). In both cases, the animals were given at least 24 h to complete the process. No other clinical findings were noted during the study. Significant reductions in mean body-weight gain were observed at 800 ppm during the period before mating and gestation (Table 47).

Hepatic enzyme activities in the treated animals were elevated at all time-points (Table 47). Estradiol levels were significantly increased at the end of the period before mating and on lactation day 2. Corticosterone levels were significantly raised at all time-points. The elevated LH and progesterone levels were possibly related to the fact that some of the animals on lactation day 2 had not delivered and were hormonally comparable with pregnant animals. No changes were detected in FSH, T4, T3, TSH, oxytocin or prolactin levels. Prostaglandin and reduced glutathione levels were not affected by treatment.

In the ovaries, no aromatase induction was detected at the end of the phase before mating or during gestation. Ovarian aromatase activity, however, was increased at 800 ppm on day 2 of lactation. In particular, levels were especially high in animals that had dystocia and did not deliver. These animals were not comparable to the control animals that had delivered and were endocrinologically in lactation. Thus, this increase in aromatase activity in the ovaries was not related to administration of thiacloprid. Increased hepatic aromatase activity was detected at 800 ppm (Table 47).

Concentrations of uterine estrogen and progesterone receptors were not affected by treatment.

Liver weight was increased in the treated animals at necropsy. Microscopy revealed centrilobular hepatocytomegaly and proliferation of the smooth endoplasmic reticulum in the liver.

Table 47. Selected findings in a special study of reproduction in rats fed diets containing thiacloprid.

Finding	Time-point	Dietary concentration (ppm)	
		0	800
Body weight	After 9 ± 1 weeks	226.9	218.7
	Day 18 of gestation	304.1	303.5
	Day 21 of gestation	358.7	323.9*
	Day 2 of lactation	271.1	244.3*
Liver weight (g) / relative liver weight (%)	After 9 ± 1 weeks	8.8/3.88	10.7*/4.88*
	Day 18 of gestation	13.0/4.28	15.1*/4.97*
	Day 21 of gestation	13.2/3.69	14.3/4.41*
	Day 2 of lactation	11.5/4.26	12.0/4.89*
Cytochrome P450 (nmol/min per mg protein)	After 9 ± 1 weeks	0.10	0.62*
	Day 18 of gestation	0.13	0.56*
	Day 2 of lactation	0.19	0.40
N-demethylase (nmol/min per mg protein)	After 9 ± 1 weeks	0.99	3.13*
	Day 18 of gestation	0.69	1.84*
	Day 2 of lactation	0.70	2.86*
O-demethylase (nmol/min per mg protein)	After 9 ± 1 weeks	0.12	0.30*
	Day 18 of gestation	0.07	0.19*
	Day 2 of lactation	0.09	0.27*
Estradiol (pg/ml)	After 9 ± 1 weeks	34.37	48.12*
	Gestation day 18	31.32	39.60
	Day 2 of lactation	19.35	48.65*
Progesterone (ng/ml)	After 9 ± 1 weeks	20.50	23.67
	Day 18 of gestation	72.73	88.70
	Day 2 of lactation	16.81	24.17*
Luteinizing hormone (ng/ml)	After 9 ± 1 weeks	0.68	1.05*
	Day 18 of gestation	0.46	0.87
	Day 2 of lactation	0.27	0.52*

Corticosterone (ng/ml)	After 9 ± 1 weeks	316.7	478.46*
	Day 18 of gestation	225.15	396.58*
	Day 2 of lactation	264.84	391.52*
Cholesterol (mg/dl)	After 9 ± 1 weeks	58	88*
	Day 18 of gestation	66	72
	Day 2 of lactation	70	78
Aromatase, ovaries (pmol/g per min)	After 9 ± 1 weeks	3.7	3.6
	Day 18 of gestation	23.1	25.0
	Day 2 of lactation	10.0	26.1*[a]
Aromatase, liver (pmol/g per min)	After 9 ± 1 weeks	7.5	14.7**

From Christenson (1998) and Schmidt (1998a)

* $p < 0.05$; ** $p < 0.01$.

[a] Two animals with the highest aromatase activities did not deliver.

The above data suggested that the adverse thiacloprid-induced effect on steroid levels resulted from an effect on the liver and that the underlying mechanism involved hepatic enzyme induction (Christenson, 1998; Schmidt, 1998a).

(f) Toxicokinetics of thiacloprid in pregnant and non-pregnant rats

In a study conducted to determine whether the toxicokinetic behaviour of thiacloprid was affected by gestation, groups of female Sprague-Dawley rats were given diets containing thiacloprid (purity, 97.2%) at a concentration of 0 or 1000 ppm during the period before mating and gestation. Eight pregnant rats and 12 non-pregnant rats received thiacloprid while five pregnant and five non-pregnant rats served as controls. Clinical observations and body weights were recorded at appropriate time-points. Blood samples were taken from the orbital plexus of anaesthetized animals on days 0, 7, 14 and 21 of gestation, and from non-pregnant females at comparable time-points. The number of live and stillborn pups born and the duration of gestation were recorded. The rats were sacrificed after giving birth. Homogeneity and stability of the test substance were known from a previous study. The concentration in the feed mixture was not analysed. No feed intake data were included in the report. The determination of thiacloprid in plasma was performed by high-performance liquid chromatography (HPLC) analysis.

No compound-related deaths occurred during the study. There were no treatment-related clinical signs of toxicity or body-weight changes. The mean duration of gestation and the number of stillborn pups were similar in control and treated groups.

The plasma concentrations of thiacloprid in non-pregnant rats on days 0, 7, 14 and 21 were 63.2, 61.8, 58.8 and 56.6 nmol/ml respectively. In pregnant rats, the plasma concentrations of thiacloprid were significantly increased during gestation and reached a peak at the end of gestation. The concentrations on days 0, 7, 14 and 21 were 57.3, 83.6, 71.1 and 85.7 nmol/ml, respectively. The higher systemic exposure in pregnant animals might explain the observed effects on birth at the end of gestation. However, other data for non-pregnant animals were consistent with the values obtained for pregnant animals (Andrews & Schmidt, 1998).

(g) In-vitro study on enzymes involved in steroid degradation

Investigations (that did not comply with GLP or guidelines) were performed in vitro to examine whether thiacloprid has any inhibiting effect on enzymes involved in the degradation of steroids. The potential inhibition of ECOD by thiacloprid was measured in liver microsomes of male rats, phenobarbital-treated male rats and male dogs. For the determination of IC_{50} values, thiacloprid was tested at concentrations of 0, 0.1, 1, 10 and 100 µmol/l. ECOD correlates with the cytochrome P450

subtypes 1A1, 2B1, 2D1 and others. Furthermore, the inhibition of testosterone hydroxylation was determined for male rats. The main hydroxylation and oxidation reactions for testosterone are catalysed by different cytochrome P450 subtypes. For the determination of the IC_{50} values, thiacloprid was tested at concentrations of 0, 10, 100, 500 and 1000 µmol/l. Liver microsomes from female rats, which were treated with thiacloprid for 2 weeks, were also used in this experiment. The main metabolites identified by HPLC were 16α, 2α, 6β and 7α hydroxylation products and androstendione.

Thiacloprid was found to be a very weak inhibitor of ECOD in all microsomal preparations. In both rats and dogs, 50% inhibition could not be achieved and the IC_{50} was > 100 µmol/l. Thiacloprid did not inhibit the main hydroxylation and oxidation reactions of testosterone. The IC_{50} for all reactions was > 1000 µmol/l. However, thiacloprid was shown to increase the metabolism of the steroid testosterone.

These tests did not reveal any inhibiting effect on enzymes involved in degradation of steroids so an increased metabolism/excretion of estradiol and compensatory feedback reactions are not involved. Induction of enzymes that catalyse the metabolism of testosterone to androstenedione was detected. Since androstenedione is a precusor of estradiol, it is possible that this pathway may contribute to the increased estradiol production in female rats (Schmidt, 1998b).

3. Observations in humans

No reports of adverse effects were identified during routine monitoring of production-plant workers and among personnel involved in experimental biological testing or field trials with thiacloprid. There was no evidence or data available to support any findings in relation to poisoning with thiacloprid.

Comments

Biochemical aspects

After oral administration to rats, [^{14}C]methylene- or [^{14}C]thiazolidine-labelled thiacloprid was rapidly and almost completely absorbed after a single low dose (1 mg/kg bw) and about 10% less well absorbed after a single high dose (100 mg/kg bw), with maximum plasma concentrations of radioactivity occurring at 1–3 h or 3–4 h, respectively. Radioactivity was widely distributed throughout the body. Tissue residues at 48 h after dosing accounted for less than 1% ([^{14}C]methylene label) or up to about 3% ([^{14}C]thiazolidine label) of the administered dose, with liver, kidneys, lung, adrenals and thyroid containing the highest residues. Excretion of radioactivity was rapid, primarily via urine (60–83%) and to a lesser extent via faeces (up to 34%) and exhaled air (< 1%), with > 90% of an administered low dose excreted within 48 h. Intravenous administration (1 mg/kg bw) revealed that the faecal radioactivity was largely due to biliary excretion. Thiacloprid was extensively metabolized and a total of 25 metabolites were identified. Metabolic transformations included *C*- and *N*-hydroxylation, *S*-oxidation and methylation, oxidative ring cleavage and methylene bridge cleavage, glucuronic acid, glycine and pentose sulfate conjugations.

Mechanistic studies showed that in males, plasma concentrations of thiacloprid reached a peak on day 8 and decreased slightly from day 15 onwards, while in females, thiacloprid concentrations reached a peak on day 8 and remained relatively constant for the duration of the study (28 days). Also in pregnant rats, concentrations of thiacloprid in plasma increased during pregnancy and reached a peak at the end of gestation.

Toxicological data

Thiacloprid was of moderate acute toxicity after oral (LD_{50}, 396–836 mg/kg bw) and inhalation (LC_{50}, 1.223 to > 2.535 mg/l) exposure in rats, with females being more sensitive than males. Thiacloprid

was of low acute dermal toxicity (LD_{50} > 2000 mg/kg bw) in rats. Thiacloprid was not a skin irritant in rabbits, it was a slight eye irritant in rabbits, and it was not a skin sensitizer in guinea-pigs.

In short-term studies of toxicity, the liver was the primary target organ of thiacloprid in rodents. In rats and mice, a dose-dependent induction of liver enzymes occurred that was associated with increased liver weight, centrilobular hypertrophy and changes in the cytoplasm of the hepatocytes. In female mice, an increase in fatty vacuolization and hypertrophy of the adrenal X-zone was also seen. Changes in circulating hormone concentrations (e.g. T4, T3 and TSH) and effects on the rat thyroid (e.g. increased weight, hypertrophy and increased mitotic rate of follicular cells) were observed as a consequence of the liver enzyme induction. Similar toxicological profiles were seen in rats exposed dermally or by inhalation. After oral and dermal administration in rats, enzyme induction and increased liver weight were shown to be reversible by the end of a 5-week or 2-week recovery period. The thyroid follicular cell hypertrophy was also shown to be at least partly reversible after dermal administration and a 2-week recovery period.

In a 14-week feeding study in mice, the NOAEL was 50 ppm, equal to 19.9 mg/kg bw, on the basis of marked vacuolization in the adrenal X zone in females at 250 ppm and higher. Effects at higher doses included liver changes (increased weight, hypertrophy of hepatocytes) in both sexes and increased adrenal weights in females at 1250 ppm and higher.

In a 2-week study in rats treated by gavage, the NOAEL was 20 mg/kg bw per day on the basis of reduced body-weight gain and reduced feed intake at 60 mg/kg bw per day. In a 2-week feeding study in rats, the NOAEL was 100 ppm, equal to 9.8 mg/kg bw per day, on the basis of decreased body weights in females and thyroid effects (increased mitosis) in males at 500 ppm and higher. In a 13-week feeding study with a 5-week recovery period in rats, the NOAEL was 400 ppm, equal to 28.6 mg/kg bw per day, on the basis of reduced body weight, clinical chemistry changes (increased cholesterol and protein concentration in plasma) and thyroid effects (increased weight) at 1600 ppm. The hypertrophy of hepatocytes in males at 1600 ppm was not completely reversible during the recovery period.

In a 5-day study in rats exposed to thiacloprid by inhalation followed by a 2-week recovery period, the NOAEC was 0.019 mg/l (equal to 4.6 mg/kg bw per day) on the basis of clinical signs, reduced body weight and decreased thymus weights at 0.205 mg/l. All effects had resolved by the end of the recovery period. In a 4-week study in rats treated by inhalation, the NOAEC was 0.018 mg/l, equal to 4.4 mg/kg bw per day, on the basis of clinical signs, reduced body weights, liver toxicity in females (increased plasma concentrations of cholesterol, alkaline phosphatase and bile acids) and thyroid effects in males (increased weight, hypertrophy of follicular epithelium) at 0.143 mg/l.

In a 4-week study in rats treated dermally, the NOAEL for systemic toxicity was 300 mg/kg bw per day on the basis of thyroid follicular cell hypertrophy at 1000 mg/kg bw per day. Hypertrophy of hepatocytes and thyroid follicular cells of males at 1000 mg/kg bw per day was partially reversible after a 2-week recovery period. The NOAEL for skin reactions was 1000 mg/kg bw per day, the highest dose tested.

In dogs, the liver was also the main target organ but enzyme induction and the subsequent changes in thyroid hormone levels were less pronounced than in rodents. In a 15-week feeding study in dogs, the NOAEL was 250 ppm, equal to 8.5 mg/kg bw per day, on the basis of reduced T4 levels, liver effects (increased weight, enzyme induction) and prostate effects (increased weight, hypertrophy of the glandular epithelium) at 1000 ppm and higher. In a 52-week feeding study in dogs, the NOAEL was 250 ppm, equal to 8.3 mg/kg bw per day, on the basis of decreased T4 levels, liver effects (hepatocellular cytoplasmic changes) and prostate effects (increased weight and size) at 1000 ppm.

Thiacloprid gave negative results in an adequate battery of studies of genotoxicity in vitro and in vivo.

The Meeting concluded that thiacloprid was unlikely to be genotoxic.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. As in short-term studies, the liver was the primary target organ, with induction of liver enzymes being the most sensitive end-point in both rats and mice. Effects on other organs, such as thyroid, adrenals, ovaries and uterus were considered to be secondary to the increased liver enzyme activities and the subsequent hormonal imbalances. The increased incidences of lens fibre degeneration and retinal atrophy in female rats at the intermediate doses (50 and 500 ppm) were not considered to be treatment-related when compared with the data for historical controls, rather they were caused by the low survival rate of females in the control group. The increased incidences of degenerative changes in the nervous system and skeletal muscles in females at 500 ppm and higher were considered to reach statistical significance owing to increased survival at the highest dose compared with the concurrent control group, resulting in a seemingly increased incidence of age-related findings.

In the study of carcinogenicity in mice, the NOAEL for systemic toxicity was 30 ppm, equal to 5.7 and 10.9 mg/kg bw per day in males and females, respectively, on the basis of effects in the liver (increased weight, hepatocellular hypertrophy, fatty change, degeneration) and lymph nodes (vacuolization) in both sexes and in the adrenal cortex (vacuolization in the X-zone) in females at 1250 ppm and higher. There was no evidence of oncogenic activity in males. In females, the incidence of benign ovarian luteomas was significantly increased at 1250 ppm and higher.

A 13-week mechanistic study on ovarian tumorigenesis in mice showed increased hepatic aromatase activity and vacuolization in the X-zone of the adrenal cortex at 250 ppm and higher, while plasma estradiol/progesterone ratio was decreased and plasma progesterone and liver weights were increased at 2500 ppm. Co-treatment with mecamylamine (a nicotine mimic) did not abolish the effects of thiacloprid on the estradiol/progesterone ratio.

The Meeting concluded that the increased incidence of ovarian luteomas in mice was a secondary consequence of liver enzyme (especially aromatase) induction, resulting in increased estradiol synthesis. Increased estradiol levels in mice but not in rats produce a positive feedback response with an increased prolactin release which consequently stimulates ovarian tissues and may explain the increased incidence of ovarian tumours. Based on application of the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans to the limited available data, the Meeting concluded that the probable mode of action for the luteomas seen in mice is exclusively a high-dose phenomenon that is not relevant for human exposure at the levels of residues found in food.

In the long-term study of toxicity and carcinogenicity in rats, the NOAEL for systemic toxicity was 25 ppm, equal to 1.2 mg/kg bw per day, on the basis of liver toxicity (increased mixed eosinophilic-clear cell foci) and thyroid effects (follicular epithelial hypertrophy) in males at 50 ppm and greater. There was also evidence of increased thyroid hyperplasia at 500 ppm and greater. The NOAEL for oncogenicity was 50 ppm, equal to 2.5 mg/kg bw per day, on the basis of the increased incidences of thyroid follicular cell adenoma in males and uterine adenocarcinoma in females at 500 ppm and greater.

Mechanistic studies on thyroid tumorigenesis in rats showed increased liver weights and increased UDP-GT activities at 400 ppm and greater, with the consequence of a decrease in T4, increase in TSH and thyroid follicular cell hypertrophy. The NOAEL was 100 ppm, equal to 9.0 mg/kg bw per day in males and females, respectively. Thiacloprid or its hydrolysis products had no inhibitory effect on thyroid peroxidase activity in vitro (IC_{50} > 870 µmol/l). Also, plasma extracts from rats treated with thiacloprid had no inhibitory effect on the activity of thyroid peroxidase in vitro.

The Meeting concluded that the increased incidence of thyroid adenoma observed in male rats was a consequence of liver enzyme induction, leading to increased clearance of thyroid hormones and increased levels of TSH. Based on application of the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans to the available data, the Meeting concluded that the mode of action for the thyroid adenomas seen in rats is exclusively a high-dose phenomenon not relevant for human exposure at the levels of residues found in food.

Mechanistic studies on uterine tumorigenesis in rats showed a dose-related induction of hepatic aromatase at 200 ppm and higher, while liver weights were increased at 500 ppm and higher. Ovarian aromatase activity was similar in control and treated groups.

The Meeting concluded that the increased incidence of uterine tumours in rats was a secondary consequence of hepatic enzyme (especially aromatase) induction, resulting in increased estradiol synthesis. Long-lasting elevations of estradiol concentrations can lead to an increased incidence of uterine adenocarcinomas especially in older rats, which become acyclic and vulnerable towards the end of their life. Based on application of the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans to the limited available data, the Meeting concluded that the probable mode of action for the uterine tumours seen in rats is exclusively a high-dose phenomenon not relevant for human exposure at the levels of residues found in food.

On the basis of the above considerations on the likely modes of action for the different tumours observed, the high doses required to induce the tumours and the negative results of studies of genotoxicity, the Meeting concluded that the increased tumour incidences associated with exposure to thiacloprid are threshold phenomena and unlikely to pose a carcinogenic risk to humans at exposure levels relevant to residues found in food.

In studies of reproductive toxicity in rats, difficulties at parturition (dystocia) were observed in some studies, with the possible consequences of increased incidence of stillbirths, decreased live birth index and/or reduced viability of pups at higher doses.

In a two-generation study of reproductive toxicity in rats, the NOAEL for parental toxicity was 50 ppm, equal to 3.5 mg/kg bw, on the basis of thyroid effects (increased weights, thyroid follicular cell hypertrophy) at 300 ppm and greater. The NOAEL for reproductive toxicity was 50 ppm, equal to 3.5 mg/kg bw, on the basis of dystocia and decreased pup weights at 300 ppm and greater, and decreased live birth index at 600 ppm.

In a supplementary one-generation study in rats, aimed at evaluating the reproducibility of dystocia and stillbirths, four dams at the highest dose of 1000 ppm died during gestation, two died after having begun birth. The incidence of stillbirths was unaffected. Pup survival and body weights were lower and the incidence of 'weak' pups was higher at 1000 ppm than in the controls. The NOAEL for systemic toxicity was 300 ppm, equal to 20 mg/kg bw per day, on the basis of clinical signs (including death), decreased body weight, and increased liver and thyroid weights at 1000 ppm. The NOAEL for reproductive toxicity was 300 ppm, equal to 20 mg/kg bw per day, on the basis of dystocia and decreased pup viability and pup weights at 1000 ppm.

Two modified one-generation studies in rats were conducted to further investigate possible causes of dystocia and stillbirths. In the study using a dose of 800 ppm, lower body weights, increased liver weights including hepatocyte hypertrophy and liver enzyme induction, increased serum steroid hormone (estradiol, progesterone, corticosterone) and luteinizing hormone levels were seen, while there were no effects on follicle-stimulating hormone, oxytocin and prolactin, on uterine and cervical prostaglandin, on liver and uterine glutathione and on uterine estrogen and progesterone levels. Aromatase activity was significantly increased in the liver (at the end of the period before mating) and in the ovaries (on day 2 of lactation). In the study using a dietary concentration of 1000 ppm, feed consumption, body weights and litter size were reduced, while there were no effects on cervical extensibility and uterine structure and function.

In a further study aimed at investigating possible dystocia, mated female rats were given thiacloprid at doses of 17.5 to 60 mg/kg bw per day by oral gavage on days 18–22 of gestation. Lower body-weight gain and feed consumption were observed in all treated groups, while clinical signs (including hypoactivity, chromorhinorrhoea, clear vaginal discharge) and deaths were seen at 35 mg/kg bw per day and higher. Dystocia was not observed, but stillbirths were higher at 35 mg/kg bw per day and higher.

The Meeting concluded that there was no evidence for a specific effect of thiacloprid on parturition. Changes in metabolism and hormonal balance were considered to be the primary cause of dystocia and stillbirths at clearly maternally toxic doses.

In a study of prenatal developmental toxicity in rats, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of decreased body weights and feed intake at 50 mg/kg bw per day. The NOAEL for developmental toxicity was 10 mg/kg bw per day on the basis of increased resorptions, reduced fetal weights, and an increase in skeletal variations at 50 mg/kg bw per day.

In a study of prenatal developmental toxicity in rabbits, the NOAEL for maternal toxicity was 2 mg/kg bw per day on the basis of decreased body weights and feed intake at 10 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 2 mg/kg bw per day on the basis of reduced fetal weights at 10 mg/kg bw per day and greater, and a higher incidence of postimplantation losses, supernumerary thirteenth ribs and incomplete ossification at 45 mg/kg bw per day.

The Meeting concluded that thiacloprid was not selectively toxic to embryo or fetal development and was not teratogenic.

In studies of acute neurotoxicity in rats, clinical signs (e.g. tremors, decreased activity, ataxia, dilated pupils, ptosis) were evident at 109 mg/kg bw, the highest dose tested, while effects on FOB observations and on motor and locomotor activity were seen at doses of 22 mg/kg bw and greater in males and 11 mg/kg bw and greater in females. All effects were generally apparent only on the day of dosing. The microscopic examinations did not reveal any lesions in the nervous system, eyes or skeletal muscle. The NOAEL for neurotoxicity was 11 mg/kg bw in males on the basis of FOB observations (slight tremors, ptosis) at 22 mg/kg bw and greater, and 3.1 mg/kg bw in females on the basis of decreased motor and locomotor activity at 11 mg/kg bw and greater.

In a 13-week study of neurotoxicity in rats, no compound-related clinical signs were observed at 1600 ppm, the highest dose tested. The FOB, the motor and locomotor activity recordings and the macroscopic and microscopic examinations did not reveal any compound-induced changes. Thus, the NOAEL for neurotoxicity was 1600 ppm, equal to 101 and 115 mg/kg bw per day in males and females, respectively. The NOAEL for systemic toxicity was 50 ppm, equal to 2.9 and 3.4 mg/kg bw per day in males and females, respectively, on the basis of reduced feed intake at 400 ppm and greater.

In a study of developmental neurotoxicity in rats, thiacloprid did not cause any specific neurobehavioural or neuropathological effects in the offspring when administered to the dams during gestation and lactation at dietary concentrations of up to 500 ppm. Non-specific signs of general toxicity, including decreased body weights and body-weight gains and delayed sexual maturation, were observed in the offspring at dietary concentrations of 300 ppm and greater. The NOAEL for maternal toxicity was 50 ppm, equal to 4.4 mg/kg bw per day, on the basis of reduced body weights and feed consumption at 300 ppm and greater. The NOAEL for toxicity to offspring was 50 ppm, equal to 4.4 mg/kg bw per day, on the basis of reduced body weights in both sexes and delayed sexual maturation (preputial separation) in males at 300 ppm and greater, and delayed sexual maturation (vaginal patency) in females at 500 ppm.

The environmental metabolites thiacloprid-amide (KKO 2254), thiacloprid-sulfonic acid sodium salt (WAK 6999), and thiacloprid-sulfonic acid amide were of low acute oral toxicity in rats (LD_{50} > 2000 mg/kg bw) and were not mutagenic in assays for reverse mutation in bacteria, in tests for gene mutation or chromosome aberration in mammalian cells.

In a short-term feeding study, rats given thiacloprid at 1000 ppm for 7 days had increased liver weights and a strong induction of liver enzymes including aromatase. Rats given the metabolites thiacloprid-sulfonic acid sodium salt (WAK 6999) and thiacloprid-sulfonic acid amide at a dose of 1000 ppm did not show these effects.

There were no reports of adverse health effects in manufacturing plant personnel or in operators and workers exposed to thiacloprid formulations during field trials. Also, there were no reports of poisonings with thiacloprid.

The Meeting concluded that the existing database on thiacloprid 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 1.2 mg/kg bw per day identified in a 2-year dietary study in rats, and a safety factor of 100. Effects at the lowest-observed-adverse-effect level (LOAEL) of 2.5 mg/kg bw per day included liver toxicity and thyroid changes (follicular epithelial hypertrophy) secondary to liver enzyme induction.

The Meeting established an ARfD of 0.03 mg/kg bw based on the NOAEL of 3.1 mg/kg bw identified in a study of acute neurotoxicity in rats, and a safety factor of 100. It was not possible to determine a chemical-specific adjustment factor since it was not confirmed by appropriate toxicokinetic data that the critical effect (decrease in motor and locomotor activity) at the LOAEL of 11 mg/kg bw is dependent on the C_{max}.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	14-week study of toxicity[a]	Toxicity	50 ppm, equal to 19.9 mg/kg bw per day	250 ppm, equal to 102.6 mg/kg bw per day
	2-year study of carcinogenicity[a]	Toxicity	30 ppm, equal to 5.7 mg/kg bw per day	1250 ppm, equal to 234.1 mg/kg bw per day
		Carcinogenicity	Females: 30 ppm, equal to 10.9 mg/kg bw per day	Females: 1250 ppm, equal to 475.3 mg/kg bw per day
Rat	13-week study of toxicity[a]	Toxicity	400 ppm, equal to 28.6 mg/kg bw per day	1600 ppm, equal to 123.2 mg/kg bw per day
	2-year study of toxicity and carcinogenicity[a]	Toxicity	25 ppm, equal to 1.2 mg/kg bw per day	50 ppm, equal to 2.5 mg/kg bw per day
		Carcinogenicity	50 ppm, equal to 2.5 mg/kg bw per day	500 ppm, equal to 25.2 mg/kg bw per day
	Multigeneration reproductive toxicity[a]	Fertility	600 ppm, equal to 43.9 mg/kg bw per day[c]	—
		Parental toxicity	50 ppm, equal to 3.5 mg/kg bw per day	300 ppm, equal to 21.7 mg/kg bw per day
		Offspring toxicity	50 ppm, equal to 3.5 mg/kg bw per day	300 ppm, equal to 21.7 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	50 mg/kg bw per day
		Embryo- and fetotoxicity	10 mg/kg bw per day	50 mg/kg bw per day
	Acute neurotoxicity[b]	Neurotoxicity	3.1 mg/kg bw per day	11 mg/kg bw per day
	Subchronic neurotoxicity[a]	Neurotoxicity	1600 ppm, equal to 101 mg/kg bw per day[c]	—
	Developmental neurotoxicity[a]	Maternal toxicity	50 ppm, equal to 4.4 mg/kg bw per day	300 ppm, equal to 25.6 mg/kg bw per day
		Offspring toxicity	50 ppm, equal to 4.4 mg/kg bw per day	300 ppm, equal to 25.6 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	2 mg/kg bw per day	10 mg/kg bw per day
		Embryo- and fetotoxicity	2 mg/kg bw per day	10 mg/kg bw per day
Dog	1-year study of toxicity[a]	Toxicity	250 ppm, equal to 8.3 mg/kg bw per day	1000 ppm, equal to 33.8 mg/kg bw per day

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

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

0.03 mg/kg bw

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

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

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

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of oral absorption	Rapid and almost complete, based on oral and intravenous administration of low dose
Dermal absorption	No data
Distribution	Widely; highest concentrations in liver, kidneys, lung, adrenals and thyroid
Rate and extent of excretion	Rapid, > 90% within 48 h; 60–83% in urine, up to 34% in faeces (largely due to biliary excretion)
Potential for accumulation	No evidence of accumulation
Metabolism in mammals	Extensive; *C*- and *N*-hydroxylation, *S*-oxidation and methylation, oxidative-ring cleavage and methylene-bridge cleavage, conjugation
Toxicologically significant compounds (animals, plants and the environment)	Parent compound
Acute toxicity	
Rat, LD_{50}, oral	396–836 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50} inhalation	1.233 to > 2.535 mg/l air (4-h, nose-only exposure)
Rabbit, skin irritation	Not irritant
Rabbit, eye irritation	Slightly irritant
Guinea-pig, skin sensitization (test method)	Not sensitizing (maximization)
Short-term studies of toxicity	
Target/critical effect	Liver (histopathological changes), thyroid (hormonal and histopathological changes), adrenals (X-zone: histopathological changes)
Lowest relevant oral NOAEL	8.3 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	300 mg/kg bw per day (4-week study in rats)
Lowest relevant inhalation NOAEC	0.002 mg/l air (4-week study in rats)
Genotoxicity	
	Not genotoxic in vitro or in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver (histopathological changes), thyroid (hormonal and histopathological changes), adrenals (X-zone: histopathological changes)
Lowest relevant NOAEL	1.2 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Thyroid adenomas in male rats, uterine adenocarcinoma in rats, ovarian luteomas in mice

Reproductive toxicity	
Reproductive target/critical effect	Dystocia; increased incidence of stillbirths, decreased live birth index, decreased pup viability and pup weights at maternally toxic doses
Lowest relevant reproductive NOAEL	43.9 mg/kg bw per day (effects on fertility at highest dose tested in a two-generation study in rats)3.5 mg/kg bw per day (systemic toxicity in offspring and parents)
Developmental target/critical effect	Increased resorptions, increased skeletal retardations and variations, decreased fetal weights at maternally toxic doses
Lowest relevant developmental NOAEL	2 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity	Clinical signs, effects in FOB observations, decreased motor and locomotor activity; NOAEL: 3.1 mg/kg bw
Subchronic neurotoxicity	No evidence of neurotoxicity; NOAEL: 101 mg/kg bw per day at highest dose tested
Developmental neurotoxicity	No evidence of developmental neurotoxicity; decreased body weight and delayed sexual maturation at maternally toxic doses; NOAEL: 4.4 mg/kg bw per day
Other toxicological studies	
Studies on metabolites	Thiacloprid-amide (KKO 2254), thiacloprid-sulfonic acid Na-salt (WAK 6999), and thiacloprid-sulfonic acid amide were of low acute oral toxicity in rats (LD_{50} > 2000 mg/kg bw) and not mutagenic in vitro
	No induction of rat liver enzymes by thiacloprid-sulfonic acid sodium salt and thiacloprid-sulfonic acid amide
Enzyme induction	Induction of hepatic cytochrome P450s, UDP-glucuronyl transferase and aromatase
Medical data	Limited data; no adverse health effects reported in manufacturing plant personnel or in operators and workers exposed during field trials

Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Rat, 2-year study	100
ARfD	0.03 mg/kg bw	Rat, study of acute neurotoxicity	100

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Appendix 1

Application of the IPCS Conceptual Framework for Cancer Risk Assessment

(IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans)

This framework, developed by an International Programme on Chemical Safety (IPCS) working group, provides a generic approach to the principles commonly used in evaluating a postulated mode of action (MOA) for tumour induction by a chemical. Thus, the framework was used by the 2006 JMPR to provide a structured approach to the assessment of the overall weight-of-evidence for the postulated mode of action for the increased incidences of thyroid follicular cell adenomas and uterine adenocarcinoma in rats and for the increased incidences of ovarian luteomas in mice observed after long-term administration of thiacloprid.

The framework guidelines suggested 10 section headings. The introduction describes the cancer end-points that have been observed. Three of these (thyroid follicular cell adenomas and uterine adenocarcinoma in rats, ovarian luteomas in mice) are addressed separately in the analysis. An appropriate mode of action is postulated and the key events identified; the observed dose–response relationships and temporal relationships are discussed. The strength, consistency, and specificity of the association of tumour response with key events and the biological plausibility are analysed. Alternative modes of action are discussed and found not to be supported. The three postulated modes of action are assessed below, with some uncertainties about both the biology of tumour development and the database on the compound and any inconsistencies in the method that were identified.

1. Thyroid follicular cell adenomas in rats

1.1 Introduction

In the long-term study of toxicity and carcinogenicity in rats, increased incidences of thyroid follicular cell adenomas were observed in males at dietary concentrations of 500 and 1000 ppm, equal to 25 or 52 mg/kg bw per day (see monograph section 2.3).

1.2 Postulated mode of action (theory of the case)

The postulated mode of action for thiacloprid-induced thyroid follicular cell tumours involves the perturbation of homeostasis of the pituitary–thyroid axis by an extra-thyroidal mechanism. Specifically, thiacloprid induces hepatic uridine diphosphate glucuronosyl transferase (UDP-GT) activity, leading to enhanced metabolism of thyroxin (T4) by conjugation and increased biliary excretion of the conjugated hormone. The pituitary gland responds to a decrease in circulating serum concentrations of T4 by enhancing the release of thyroid stimulating hormone (TSH). Prolonged elevation of circulating TSH concentrations stimulates the thyroid gland to increase thyroid hormone synthesis and release, thus leading to thyroid follicular cell hypertrophy and hyperplasia. With long-term exposure, thyroid hyperplasia eventually progresses to neoplasia.

1.3 Key events

The sequence of key events in the mode of carcinogenic action of thiacloprid in the thyroid includes:

- induction of hepatic UDP-GT activity;
- increase in hepatic metabolism and biliary excretion of T4;
- decrease in serum T4 half life and concentration;
- increase in serum TSH concentration;
- thyroid follicular cell hypertrophy and hyperplasia.

The key events as described above include changes in liver metabolism, alterations in hormone levels, increase in thyroid growth, and lesion progression in the thyroid. These effects have been investigated and observed in male rats in short-term and/or mechanistic studies, and at interim and terminal sacrifices in a long-term study. The dose–response relationship and the temporal analyses of the key events and tumour response are presented below.

1.4 Concordance of dose–response relationships

The no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) for the key effects in the mode of action of thiacloprid in the thyroid are provided in Table A1.

In all studies evaluated, hepatocellular hypertrophy appeared to be the most sensitive indicator of changes in liver metabolism, occurring at doses of 2.5 mg/kg bw per day and higher. A biologically significant increase in hepatic UDP-GT activity was observed in a 3-week study and in a 2-year study at doses of 25 or 37 mg/kg bw per day and higher, respectively. Consistent with the enhanced clearance of T4, decreases in serum concentrations of T4 were seen in the 3-week study at the highest dose tested (145 mg/kg bw per day), while subsequent increases in TSH were found in the 3-week study and in the 2-year study at doses of 25 or 37 mg/kg bw per day and higher, respectively. Prolonged stimulation of TSH leads to follicular cell hypertrophy and hyperplasia of the thyroid. In the 2-year study in rats, thyroid hypertrophy was seen at doses of 2.5 mg/kg bw per day and higher, while thyroid hyperplasia and increased thyroid tumour incidence were found at doses of 25 mg/kg bw per day and higher.

Generally, there was a good correlation between the doses causing induction of liver enzymes and subsequent effects on T4 and TSH with those causing an increased incidence of thyroid hyperplasia and thyroid tumours.

Table A1. NOAELs and LOAELs for key effects in the mode of action of thiacloprid in the thyroid

Effect	NOAEL/LOAEL
Liver	
Induction of UDP-GT	9/37 mg/kg bw per day (3-week mechanistic study)
	2.5/25 mg/kg bw per day (2-year study)
Increase in liver weight	37/145 mg/kg bw per day (3-week mechanistic study)
	25/52 mg/kg bw per day (2-year study)
Hepatocellular hypertrophy	1.2/2.5 mg/kg bw per day (2-year study)
Hormones	
Decrease in serum T4	37/145 mg/kg bw per day (3-week mechanistic study)
Increase in serum TSH	9/37 mg/kg bw per day (3-week mechanistic study)
	2.5/25 mg/kg bw per day (2-year study)
Thyroid	
Increase in thyroid weight	29/123 mg/kg bw per day (13-week study)
Increase in thyroid hypertrophy	9/37 mg/kg bw per day (3-week mechanistic study)
	1.2/2.5 mg/kg bw per day (2-year study)
Increase in thyroid hyperplasia	2.5/25 mg/kg bw per day (2-year study)
Increase in thyroid tumours	2.5/25 mg/kg bw per day (2-year study)

T3, triiodothyronine; T4, thyroxin; TBC, thyroxin-binding capacity; UDP-GT, uridine diphosphate glucuronosyl transferase.

1.5 Temporal association

The key events, such as induction of hepatic UDP-GT activity, increased clearance of thyroid hormones (decreased T3 and/or T4 concentrations), increased TSH concentrations and thyroid follicular cell hypertrophy were observed after a 2-week or 3-week exposure to thiacloprid. In the 2-year study I rats, the first thyroid adenoma was observed at interim sacrifice (week 52) at 52 mg/kg bw per day, the highest dose tested. Thus, there was a logical temporal response with all key events preceding tumour formation.

1.6 Strength, consistency and specificity of association of tumour response with key events

Based on information from the studies described in the monograph, there is sufficient weight of evidence that the key events (as induction of hepatic UDP-GT activity, decrease in T4, increase in TSH) are linked to the precursor lesions in the thyroid (as follicular cell hypertrophy/hyperplasia) and the ultimate tumour response. The key events were observed consistently in a number of studies with differing experimental designs. There was also evidence that the key events (such as liver enzyme induction, increased liver weight) were reversible after cessation of exposure to thiacloprid.

1.7 Biological plausibility and coherence

The relationship between sustained perturbation of the hypothalamus–pituitary–thyroid axis, prolonged stimulation of the thyroid gland by TSH, and the progression of thyroid follicular cells to hypertrophy, hyperplasia and eventually neoplasia is considered to be biologically plausible and has been shown in several studies in laboratory rats. Increased secretion of TSH may result via different mechanisms including increased hepatic clearance of T4, as is the case with thiacloprid.

The tumour response elicited by thiacloprid is typical of a rodent thyroid carcinogen in that thyroid follicular cell tumours are found in male rats but not in female rats or mice. Rats tend to be more sensitive to thyroid carcinogenesis than mice, and male rats are frequently found to be more

sensitive than female rats with respect to the proportion of chemicals that induce thyroid tumours. Consistent with this, TSH levels are typically higher in male rats than in females.

1.8 Other modes of action

Genotoxicity is always one possible mode of action to consider, but no genotoxic potential was demonstrated for thiacloprid in the following tests:

- Mutation in four strains of *Salmonella typhimurium;*
- Mutation at the *Hprt* locus of Chinese hamster V79 cells;
- Chromosomal aberration in Chinese hamster V79 cells;
- Unscheduled DNA synthesis induction in primary rat hepatocytes; and
- Micronucleus induction in bone-marrow cells of mice treated in vivo.

Therefore, the available evidence indicates that genotoxicity is not an alternative mode of action for thiacloprid.

In addition, a possible direct effect of thiacloprid on thyroid peroxidase (TPO) was considered. However, a special study confirmed that thiacloprid had no direct effect on TPO in vitro. Also, plasma extracts from thiacloprid-treated rats had no inhibitory effect on thyroid peroxidase activity in vitro.

1.9 Uncertainties, inconsistencies, and data gaps

No inconsistencies or data gaps were identified in the database for thiacloprid with regard to the postulated mode of action for thyroid follicular cell tumours.

1.10 Assessment of postulated mode of action

The data presented are considered, with a high degree of confidence, to be adequate to explain that thiacloprid exerts its carcinogenic effect on the rat thyroid secondary to enhanced hepatic clearance of thyroid hormones leading to increased secretion of TSH and enhanced thyroid growth.

1.11 Conclusion

There is sufficient experimental evidence that thiacloprid induces thyroid follicular cell tumours in rats by a process including increased hepatic clearance of thyroid hormones and disruption of the hypothalamus–pituitary–thyroid axis. Although the postulated mode of action could theoretically operate in humans, the particular sensitivity of rats for neoplasia owing to alterations in thyroid homeostasis allows for the conclusion that thiacloprid does not pose a carcinogenic risk to humans at exposure levels relevant to residues in food.

2. Uterine adenocarcinoma in rats

2.1 Introduction

In the long-term study of toxicity and carcinogenicity in rats, increased incidences of uterine adenocarcinomas were observed in females at dietary concentrations of 500 and 1000 ppm, equal to 36 or 69 mg/kg bw per day (see monograph section 2.3).

2.2 Postulated mode of action (theory of the case)

The postulated mode of action for thiacloprid-induced uterine adenocarcinomas involves hormonal disruption caused secondarily by effects on the liver. Specifically, thiacloprid is a strong inducer of hepatic microsomal cytochrome P450 enzymes, including aromatase, a key enzyme in the synthesis of estrogens that catalyses the conversion of testosterone to estradiol and of androstendione to estrone. The consequence is an increase in plasma estradiol levels and a stimulation of the uterine endometrium over the entire lifetime, which may result in an augmentation of the spontaneous incidence of uterine adenocarcinomas, especially in old and acyclic rats.

2.3 Key events

The sequence of key events in the mode of carcinogenic action of thiacloprid in the uterus includes:

- induction of hepatic aromatase activity,
- increased conversion of testosterone to estradiol and of androstendione to estrone,
- increase in serum estradiol concentration,
- long-lasting stimulation of the uterine endometrium.

The key events described above include changes in liver enzyme activities and alterations in steroid hormone levels. These effects have been investigated and observed in female rats in short-term mechanistic studies, while an increased incidence of uterine tumours was seen at terminal sacrifice in a long-term study. The dose–response relationship and the temporal analyses of the key events and tumour response are presented below.

2.4 Concordance of dose–response relationships

The NOAELs and LOAELs for the key effects in the mode of action of thiacloprid in the uterus are provided in Table A2.

In all the studies evaluated, hepatocellular hypertrophy appeared to be the most sensitive indicator of changes in liver metabolism, occurring at doses of 2.5 mg/kg bw per day and higher. A biologically significant increase in hepatic aromatase activity was observed in a 4-week mechanistic study at doses of 20.4 mg/kg bw per day and higher, and at higher doses in two further mechanistic studies. Consistent with the augmented conversion of testosterone to estradiol and of androstendione to estrone, increases in serum estradiol levels were seen in a one-generation study at 60.4 mg/kg bw per day, the only dose tested in this study. Prolonged stimulation of the endometrium by estradiol may result in an augmentation of the spontaneous incidence of uterine tumours. In the 2-year study in rats, an increased incidence of uterine adenocarcinomas was seen at doses of 25 mg/kg bw per day and higher.

Generally, there was good correlation between the doses causing induction of hepatic aromatase activity and those causing an increased incidence of uterine tumours. However, there appeared to be a lack of dose-concordance for uterine tumours and increases of serum estradiol levels, but this was obviously due to the poor selection of dose levels and/or end-points investigated in the mechanistic studies.

Table A2. NOAELs and LOAELs for key effects in the mode of action of thiacloprid in the thyroid

Effect	NOAEL/LOAEL
Liver	
Induction of hepatic aromatase	—/113 mg/kg bw per day (7-day mechanistic study)
	—/60.4 mg/kg bw per day (one-generation study)
	6.6/20.4 mg/kg bw per day (4-week mechanistic study)
Increase in liver weight	20.4/47.5 mg/kg bw per day (4-week mechanistic study)
	34/69 mg/kg bw per day (2-year study)
Hepatocellular hypertrophy	1.2/2.5 mg/kg bw per day (2-year study)
Hormones	
Increase in serum estradiol	—/60.4 mg/kg bw per day (one-generation study)
Uterus	
Increase in uterine tumours	3.3/34 mg/kg bw per day (2-year study)

2.5 Temporal association

The key events, such as induction of hepatic aromatase activity and increase in serum estradiol levels were already observed after a 7-day or 9-week exposure to thiacloprid. In the 2-year study in rats, the first uterine adenocarcinomas were observed at weeks 88, 77 or 67 at doses of 0, 34 or 69 mg/kg bw per day, respectively. Thus, there was a logical temporal response with all key events preceding tumour formation.

2.6 Strength, consistency and specificity of association of tumour response with key events

Based on information from the studies described in the monograph, the weight of evidence that the key events (as induction of hepatic aromatase activity, increase in serum estradiol level) are linked to the uterine adenocarcinomas in rats was considered to be sufficient. The key events were observed consistently in a number of studies with differing experimental designs. There was also evidence that the key events (such as liver enzyme induction, increased liver weight) were reversible after cessation of exposure to thiacloprid.

2.7 Biological plausibility and coherence

The relationship between prolonged stimulation of the uterine endometrium by increased serum estrogen levels and an increased incidence of uterine adenocarcinomas is considered to be biologically plausible and has been shown in some studies in laboratory rats. Increased serum estrogen levels may result via different mechanisms including hepatic aromatase induction, as is the case with thiacloprid. In rats that are normally exposed to relatively low estradiol levels, even a slight increase in estradiol over a longer period of time may lead to a disruption of the estrous cycle. In contrast, humans are able to handle much higher levels of estrogens than rodents.

2.8 Other modes of action

Genotoxicity is always one possible mode of action to consider, but no genotoxic potential was demonstrated for thiacloprid in the following tests:

- Mutation in four strains of *Salmonella typhimurium;*
- Mutation at the *Hprt* locus of Chinese hamster V79 cells;

- Chromosomal aberration in Chinese hamster V79 cells;
- Unscheduled DNA synthesis induction in primary rat hepatocytes; and
- Micronucleus induction in bone-marrow cells of mice treated in vivo.

Therefore, the available evidence indicated that genotoxicity is not an alternative mode of action for thiacloprid.

Tests in vitro did not reveal any inhibiting effect on enzymes involved in steroid degradation, so that an accumulation of estradiol was not the reason for the changes in serum hormone levels. However, there was an induction of enzymes which catalyse the conversion of testosterone to androstendione, which may have contributed to increased production of estradiol.

2.9 Uncertainties, inconsistencies, and data gaps

No inconsistencies were identified in the database for thiacloprid with regard to the postulated mode of action for uterine adenocarcinomas in rats. However, the data indicating an increase in serum estradiol levels as a result of exposure to thiacloprid are limited since this end-point was investigated only in a mechanistic study.

2.10 Assessment of postulated mode of action

The data presented are considered, with a moderate degree of confidence, to be adequate to explain that thiacloprid exerts its carcinogenic effect on the rat uterus secondary to induction of hepatic aromatase activity leading to increased serum estradiol levels and prolonged uterine endometrium stimulation.

2.11 Conclusion

There is experimental evidence that thiacloprid induces uterine adenocarcinomas in rats by a process including induction of hepatic aromatase and prolonged stimulation of the uterine endometrium by increased concentrations of serum estrogen. Although the postulated mode of action could theoretically operate in humans, the particular sensitivity of rats for neoplasia owing to increased estradiol levels over a longer period of time allows for the conclusion that thiacloprid does not pose a carcinogenic risk to humans at exposure levels relevant to residues in food.

3. Ovarian luteomas in mice

3.1 Introduction

In the long-term study of carcinogenicity in mice, increased incidences of ovarian luteomas were observed in females at dietary concentrations of 1250 and 2500 ppm, equal to 475 or 873 mg/kg bw per day (see monograph section 2.3).

3.2 Postulated mode of action (theory of the case)

The postulated mode of action for thiacloprid-induced ovarian luteomas involves hormonal disruption caused secondarily by effects on the liver. Specifically, thiacloprid is a strong inducer of hepatic microsomal cytochrome P450 enzymes, including aromatase, a key enzyme in the synthesis

of estrogens that catalyses the conversion of testosterone to estradiol and of androstendione to estrone. The consequence is an increase in plasma estradiol levels causing in mice, but not in rats, an increased prolactin release via positive feedback, which explains the increased incidences of luteomas rather than uterine tumours, as in rats.

3.3 Key events

The sequence of key events in the mode of carcinogenic action of thiacloprid in the ovaries includes:

- Induction of hepatic aromatase activity;
- Increased conversion of testosterone to estradiol and of androstendione to estrone;
- Increase in serum estradiol concentrations;
- Increased prolactin release via positive feedback; and
- Long-lasting stimulation of the ovarian tissues by estradiol and prolactin.

The key events as described above include changes in liver enzyme activities and alterations in steroid hormone levels. Some of these effects, such as induction of hepatic aromatase activity, have been investigated and observed in female mice in a short-term mechanistic study, while an increased incidence of ovarian luteomas was seen at terminal sacrifice in a long-term study. The dose–response relationship and the temporal analyses of the key events and tumour response are presented below.

3.4 Concordance of dose–response relationships

The NOAELs and LOAELs for the key effects in the mode of action of thiacloprid in the ovaries are provided in Table A3.

In all the studies evaluated, a dose-related increase in vacuolization of the X-zone in the adrenal cortex of female mice appeared to be the most sensitive indicator of hormonal imbalances attributable to induction of microsomal liver enzymes, occurring at doses of 139 mg/kg bw per day and higher in a 14-week study and in a 13-week mechanistic study. Hepatocellular hypertrophy was seen at doses of 475 mg/kg bw per day and higher in a 2-year study and at 704 mg/kg bw per day and higher in a 14-week study. A biologically significant increase in hepatic aromatase activity was observed in a 13-week mechanistic study at doses of 139 mg/kg bw per day and higher. Consistent with the augmented conversion of testosterone to estradiol and of androstendione to estrone, changes in the serum estradiol/progesterone ratio were seen in a 13-week mechanistic study at a dose of 1101 mg/kg bw per day. However, an increase in estradiol levels could not be detected since there was only one determination after 13 weeks of exposure. In mice, unlike in rats, an increase in plasma estradiol levels causes an increased prolactin release via positive feedback. Prolonged stimulation of ovarian tissues by estradiol and prolactin may result in hyperplastic and neoplastic proliferations. In the 2-year study in mice, an increased incidence of ovarian luteomas was seen at doses of 475 mg/kg bw per day and higher.

Generally, there was a good correlation between the doses causing induction of hepatic aromatase activity and those causing an increased incidence of ovarian tumours. However, there appeared to be a lack of dose concordance for ovarian tumours and increases of serum estradiol and/or prolactin levels, but this was obviously due to the poor selection of sampling times and/or end-points investigated in the mechanistic study.

Table A3. NOAELs and LOAELs for key effects in the mode of action of thiacloprid in the thyroid

Effect	NOAEL/LOAEL
Liver	
Induction of hepatic aromatase	18/139 mg/kg bw per day (13-week mechanistic study)
Increase in liver weight	139/704 mg/kg bw per day (14-week study)
	11/475 mg/kg bw per day (2-year study)
Hepatocellular hypertrophy	139/704 mg/kg bw per day (14-week study)
	11/475 mg/kg bw perday (2-year study)
Hormones	
Decrease in serum estradiol/progesterone ratio	139/1101 mg/kg bw per day (13-week mechanistic study)
Increase in serum progesterone	139/1101 mg/kg bw per day (13-week mechanistic study)
Ovary	
Decreased No. of old corpora lutea, activation of interstitial cells	139/704 mg/kg bw per day (14-week study)
Increased No. of large, eosinophilic and luteinized cells	11/475 mg/kg bw per day (2-year study)
Increase in luteomas	11/475 mg/kg bw per day (2-year study)

3.5 Temporal association

The key events, such as induction of hepatic aromatase activity and changes in serum estradiol and/or progesterone levels (estradiol/progesterone ratio) were observed after a 13-week exposure to thiacloprid. Increased estradiol and prolactin levels should already have been detected after shorter exposure times; however, no earlier sampling times were included in the mechanistic study. In the 2-year study in mice, the first ovarian luteomas were observed at weeks 107 or 53 at doses of 475 or 873 mg/kg bw per day, respectively. Thus, there was a logical temporal response, with the key events preceding tumour formation.

3.6 Strength, consistency and specificity of association of tumour response with key events

Based on information from the studies described in the monograph, the weight of evidence that the key events (as induction of hepatic aromatase activity, increase in serum estradiol and prolactin levels, precursor lesions in the ovarian tissues) are linked to the ovarian luteomas in mice was considered to be acceptable. Some of the key events (as induction of microsomal liver enzymes, hepatocellular hypertrophy, precursor lesions in the ovarian tissues) were observed consistently in studies with differing experimental designs, while induction of hepatic aromatase activity and changes in serum steroid hormone levels were investigated only in a mechanistic study.

3.7 Biological plausibility and coherence

The relationship between prolonged stimulation of ovarian tissues by increased serum estrogen and prolactin levels and an increased incidence of ovarian luteomas was considered to be biologically plausible. This postulated mode of action would also give an explanation for the increase of hypertrophy and vacuolization of the X-zone in the adrenal cortex of female mice, as observed in the 13-week mechanistic study, the 14-week study and the 2-year study, since it has been shown that lipid vacuolization of the X-zone is influenced by pituitary and gonadal function of mice.

3.8 Other modes of action

Genotoxicity is always one possible mode of action to consider, but no genotoxic potential was demonstrated for thiacloprid in the following tests:

- Mutation in four strains of *Salmonella typhimurium;*
- Mutation at the *Hprt* locus of Chinese hamster V79 cells;
- Chromosomal aberration in Chinese hamster V79 cells;
- Unscheduled DNA synthesis induction in primary rat hepatocytes; and
- Micronucleus induction in bone-marrow cells of mice treated in vivo.

Therefore, the available evidence indicates that genotoxicity is not an alternative mode of action for thiacloprid.

3.9 Uncertainties, inconsistencies, and data gaps

No inconsistencies were identified in the database for thiacloprid with regard to the postulated mode of action for ovarian luteomas in mice. However, there appears to be a lack of data for increases of serum estradiol and/or prolactin levels, since these endpoints have not been investigated at appropriate sampling times.

3.10 Assessment of postulated mode of action

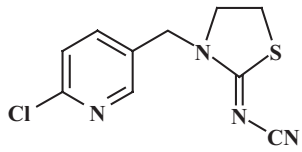
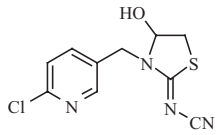
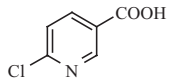
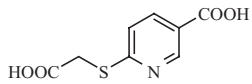
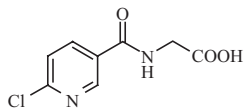
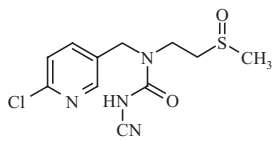
The data presented are considered, with a moderate degree of confidence, to be adequate to explain that thiacloprid exerts its carcinogenic effect on the mouse ovaries secondary to induction of hepatic aromatase activity leading to increased serum estradiol and prolactin levels and prolonged stimulation of ovarian tissues.

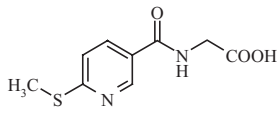
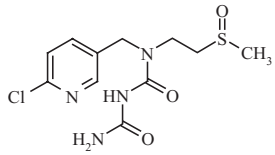
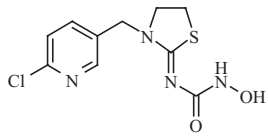
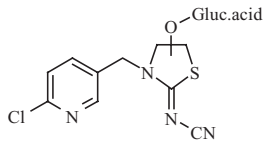
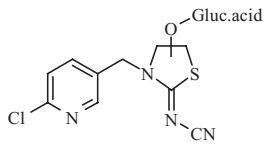
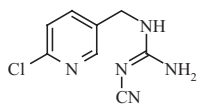
3.11 Conclusion

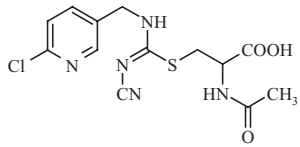
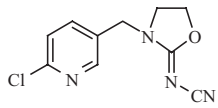
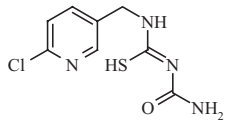
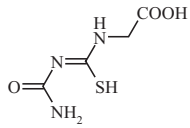
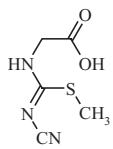
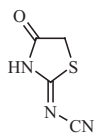
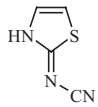
There is experimental evidence that thiacloprid induces ovarian luteomas in mice by a process including induction of hepatic aromatase and prolonged stimulation of the ovarian tissues by increased serum estrogen and prolactin levels. Although the postulated mode of action could theoretically operate in humans, the available information allows for the conclusion that induction of ovarian luteomas in mice by thiacloprid is exclusively a high dose phenomenon not relevant for human exposure at levels of residues in food.

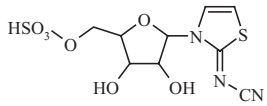
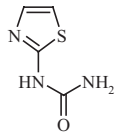
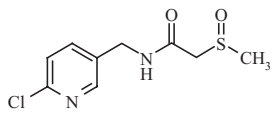
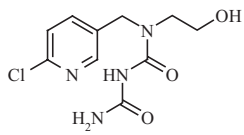
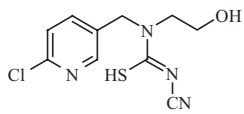
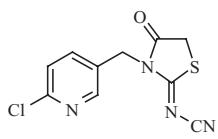
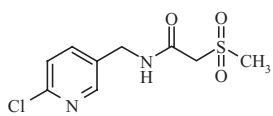
Appendix 2

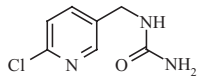
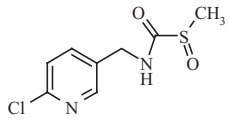
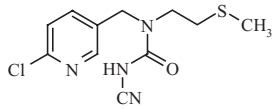
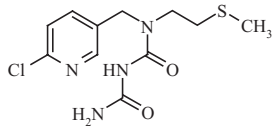
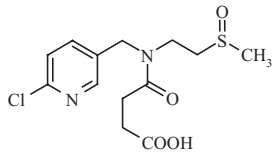
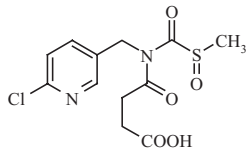
Table B1. List of animal metabolites (rat and livestock) including the different names, short forms and code numbers as used in the corresponding study reports

Metabolite	Structure / trivial name / chemical name *[CAS No.]*	Codes used	Found in
as	 YRC 2894 {3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene}cyanamide *[111988-49-9]*	YRC 2894 PIZ 1264 ECW 10874 THS 4432 Ja752-J	Apple Tomato Cotton Goat Hen Rat Soil Water sed. aerobic Water sed. anaerobic
M01	 4-hydroxy-YRC 2894 3-(6-chloro-3-pyridin-3-ylmethyl)-4-hydroxy-thiazolidine-2-ylidene-cyanamide {3-[(6-chloro-3-pyridinyl)methyl]-4-hydroxy-2-thiazolidinylidene}cyanamide	WAK 6856 PIZ 1265 KNO 1863 FHW 0106E G1	Apple Tomato Cotton Goat Hen Rat
M03	 6-CNA 6-chloronicotinic acid 6-chloro-3-pyridinecarboxylic acid *[5326-23-8]*	BNF 5518A 6-CNA Ja752-K G6	Tomato Cotton Goat Hen Rat Soil Rotational crop
M06	 6-CMT-nicotinic acid 6-[(carboxymethyl)thio]-3-pyridinecarboxylic acid	KNO 1886	Rat
M07	 6-CN-glycine *N*-[(6-chloro-3-pyridinyl)carbonyl]glycine	WAK 3583	Rat Goat Hen
M08	 6-CP-urea sulfoxide *N*-[(6-chloro-3-pyridinyl)methyl]-N'-cyano-N-[2-(methylsulfinyl)ethyl]urea	KNO 2672	Rat Goat Hen

Metabolite	Structure / trivial name / chemical name *[CAS No.]*	Codes used	Found in
M09	 Methylthionicotinoylglycine *N*-{[6-(methylthio)-3-pyridinyl]carbonyl}glycine	KNO 1889	Rrat Hen
M10	 6-CP-biuret sulfoxide *N*-[(6-chloro-3-pyridinyl)methyl]-*N*-*N*-[2-(methylsulfinyl)ethyl] iminodicarbonic diamide	KNO 1891 KNO 1873B	Rat Goat Hen
M11	 YRC 2894-hydroxylamide *N*-{3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene}-*N'*-hydroxyurea	KNO 1893	Rat Goat Hen
M12	 Glucuronic acid conjugate of hydroxy-YRC 2894 Glucuronic acid conjugate of 3-(6-chloro-pyridin-3-ylmethyl)-4-hydroxy-thiazolidine-2-ylidene-cyanamide Glucuronic acid conjugate of {3-[(6-chloro-3-pyridinyl)methyl]-4(or 5)-hydroxy-2-thiazolidinylidene}=cyanamide	KNO 2621 PIZ 1270	Rat Goat
M13	 Isomer of M12 Glucuronic acid conjugate of {3-[(6-chloro-3-pyridinyl)methyl]-5(or 4)-hydroxy-2-thiazolidinylidene}=cyanamide	KNO 2665 PIZ 1271	Rat Goat
M14	 6-CP-cyanoguanidine *N*-[(6-chloro-3-pyridinyl)methyl]-*N'*-cyanoguanidine	KNO 1872	Rat Hen

Metabolite	Structure / trivial name / chemical name *[CAS No.]*	Codes used	Found in
M15		KNO 2684	Rat Goat Hen
	S-(6-CP-cyanoamidino)-acetylcystein *N*-acetyl-3-{[N-[(6-chloro-3-pyridinyl) methyl]-*N*'-cyano]amidinothio}alanine		
M16		NTN 35078 PIZ 1266 KNO 1859	Rat Goat Hen
	YRC 2894 O-analogue 3-(6-chloro-pyridin-3ylmethyl)-oxazolidin-2-ylidene-cyanamide {3-[(6-chloro-3-pyridinyl)methyl]-2-oxazolidinylidene}cyanamide *[111988-68-2]*		
M17		KNO 1864	Rat Goat
	6-CP-thiobiuret 1-[(6-chloro-3-pyridinyl)methyl]-2-thiobiuret		
M18		PIZ 1241C	Rat
	1-CM-2-thiobiuret *N*-{[(aminocarbonyl)amino]=thioxomethyl}glycine *[51597-15-0]*		
M19		PIZ 1252 PIZ 1250	Rat
	N[(cyanoamino)(methylthio)methylene]-glycine *N*-[cyanimino(methylthio)methyl]=glycine		
M20		PIZ 1297B	Rat
	2-cyanimino-thiazolidin-4-one (4-oxo-2-thiazolidinylidene)=cyanamide *[176529-83-2]*		
M21		PIZ 1245	Rat
	3H-thiazol-2-ylidene-cyanamide 2-thiazolylcyanamide *[41227-90-1]*		

Metabolite	Structure / trivial name / chemical name *[CAS No.]*	Codes used	Found in
M22	 Sulfuric acid mono-[5-(2-cyanoimino-thiazol-3yl)-3,4-dihydroxy-tetrahydro-furan-2-ylmethyl]ester [3-(5-O-sulfono-furanosyl)-2-thiazolyl]cyanamide	PIZ 1243 PIZ 1244	Rat
M23	 Thiazol-2-yl-urea 2-thiazolylurea *[35107-91-6]*	PIZ 1249	Rat
M24	 *N*-[(6-chloro-3-pyridinyl)methyl]-2-(methylsulfinyl)acetamide	PIZ 1297E	Rat
M25	 3-aminocarbonyl-1-(6-chloro-pyridin-3-ylmethyl)-1-(2-hydroxyethyl)-urea *N*-[(6-chloro-3-pyridinyl)methyl]-*N*-(2-hydroxyethyl)imidodicarbonic diamide	WAK 6935 PIZ 1297F	Rat
M26	 1-(6-Chloro-pyridin-3-ylmethyl)-3-cyano-1-(2-hydroxyethyl)-2-thiourea *N*-[(6-chloro-3-pyridinyl)methyl]-*N'*-cyano-N-(2-hydroxyethyl) thiourea	PIZ 1253	Rat
M27	 {3-[(6-chloro-3-pyridinyl)methyl]-4-oxo-2-thiazolidinylidene} cyanamide	PIZ 1297D	Rat
M28	 *N*-(6-Chloro-pyridin-3-ylmethyl)-2-methanesulfonyl-acetamide *N*-[(6-chloro-3-pyridinyl)methyl]-2-(methylsulfonyl)acetamide	PIZ 1269X	Rat

Metabolite	Structure / trivial name / chemical name *[CAS No.]*	Codes used	Found in
M31		DIJ 10739 Ja752-H	Soil Rotational crop
	YRC 2894 urea [(6-chloro-3-pyridinyl)methyl]urea *[200258-66-8]*		
M41		KNO 2673	Goat Hen
	N-[(6-chloro-3-pyridinyl)methyl]-(methylsulfinyl)carboxamide		
M42		ANC 1502	Goat
	N-[(6-chloro-3-pyridinyl)methyl]-*N'*-cyano-*N*-[2-(methylthio) ethyl]urea		
M43		ANC 1503	Goat
	N-[(6-chloro-3-pyridinyl)methyl]-*N*-[2-(methylthio)ethyl]imi-dodicarbonic diamide		
M44		ANC 1508A	Goat
	4-{[(6-chloro-3-pyridinyl)methyl] [2-(methylthio)ethyl]amino}-4-oxo-butanoic acid		
M45		ANC 1508B	Goat
	4-{[(6-chloro-3-pyridinyl)methyl] [(methylsulfinyl)carbonyl] amino}-4-oxo-butanoic acid		

THIOPHANATE-METHYL (addendum)

First draft prepared by
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Explanation

Thiophanate-methyl is the International Organization of Standardization (ISO) approved common name for dimethyl 4,4'-(*o*-phenylene)bis(3-thioallophanate), a systemically active benzimidazole fungicide that inhibits the synthesis of β-tubulin. Thiophanate-methyl was previously evaluated by the Joint Meeting on Pesticide Residues (JMPR) in 1973, 1975, 1977, 1995 and 1998. In 1998, an acceptable daily intake (ADI) of 0–0.08 mg/kg bw was established based on the no-observed-adverse-effect level (NOAEL) of 8 mg/kg bw per day in a three-generation study of reproductive toxicity in rats and in a 1-year study in dogs (both of these studies having been evaluated at earlier meetings) and a safety factor of 100. The 1998 JMPR also concluded that an acute reference dose (ARfD) was not required because thiophanate-methyl is of low acute toxicity when administered orally or dermally and that the acute intake of residues is unlikely to present a risk to consumers.

The Meeting was asked by the Codex Committee on Pesticide Residues (CCPR) to reconsider the need for an ARfD for thiophanate-methyl. The present Joint Meeting therefore evaluated relevant original studies that had been considered by previous Meetings, and newly submitted studies on genotoxicity, reproductive toxicity, developmental toxicity and studies of acute neurotoxicity and short-term studies of neurotoxicity.

Evaluation for acute reference dose

1. Toxicological studies

1.1 Short-term studies of toxicity

Dogs

In a short-term study of toxicity conducted in compliance with the test guidelines of the United States Environmental Protection Agency (EPA), groups of four male and four female beagle dogs (age approximately 6 months at the start of the study) received gelatin capsules containing thiophanate-methyl (purity, 96.55%) at a dose of 0, 8, 40 or 200 mg/kg bw per day 1–2 h after the daily feed of 400 g, for 1 year. Physical observations, ophthalmic examinations, body weight and feed consumption measurements, haematology, biochemistry and urine analysis were performed on all animals before the test and at selected intervals during the treatment period. At sacrifice, selected organs were weighed, and all animals underwent extensive gross and histopathological examinations.

There were no deaths during the study. Tremors were seen in all dogs at 200 mg/kg bw per day approximately 2–4 h after treatment on one or more occasions during the initial 17 days of the study but not subsequently. The tremors were generally slight, except in one animal which had severe tremors that progressed to apparent tonic convulsions on three occasions (Table 1).

Table 1. Incidence of tremor in dogs given capsules containing thiophanate-methyl for 1 year

Dose (mg/kg bw per day)	Animal No.	Sex	Days of study on which tremors were observed	
			Slight tremors	Severe tremors/tonic convulsions
40	3604	Female	13	—
200	4101	Male	1, 7, 13	—
200	4102	Male	1	—
200	4103	Male	1, 4, 6, 7, 13	—
200	4104	Male	1, 7, 12, 13	—
200	4601	Female	1, 7	—
200	4602	Female	1	—
200	4603	Female	1, 2, 13, 16, 17	2, 16, 17
200	4604	Female	4	—

From Auletta (1992)

The mean body weights and body-weight gains of animals at 200 mg/kg bw per day were reduced throughout the study, and body-weight losses were noted in the first week of treatment. Two animals (male No. 4103, female No. 4604) exhibited further losses up to 0.6 kg at week 9 or 1.7 kg at week 15, respectively. Slight reductions in body-weight gain (about 81% of that of controls) were noted at 40 mg/kg bw per day. The feed consumption of dogs at the highest dose was generally lower than that of controls during the early months, but was close to the control value thereafter. Ophthalmological examinations and urine analyses showed no treatment-related effects. Haematological effects consisted of slight decreases in total erythrocyte counts and haemoglobin and erythrocyte volume fraction in males at 200 mg/kg bw per day (Table 2).

Table 2. Selected findings in dogs given capsules containing thiophanate-methyl for 1 year

Parameter	Dose (mg/kg bw per day)							
	Males				Females			
	0	8	40	200	0	8	40	200
Body weight (kg), month 12	13.0	13.7	12.1	10.4	10.7	12.4	9.9	8.5
Body-weight gain (kg), months 0–12	5.3	5.6	4.3	2.9	3.6	5.1	2.9	1.3*
Erythrocyte count (T/l), month 12	7.62	7.76	7.29	6.63**	7.61	7.23	7.35	7.25
Haemoglobin (g/dl), month 12	17.4	17.5	16.0	14.9**	16.9	16.1	16.8	16.6
Erythrocyte volume fraction, month 12	0.523	0.531	0.485	0.451**	0.518	0.489	0.518	0.514
Alkaline phosphatase (IU/l), month 12	50	35	76	150**	71	55	55	129
Cholesterol (mg/dl), month 12	166	170	216	236	169	232	210	298*
Albumin/globulin ratio, month 12	1.3	1.2	1.0*	0.9*	1.4	1.6	1.4	1.3
T3 (ng/ml)	1.09	1.19	1.19	1.06	1.14	1.37	1.40	1.70
T4 (µg/dl)	1.95	2.07	1.66	0.84*	1.96	2.23	2.42	2.07
TSH (ng/ml)	3.39	4.48	4.91	10.13	1.65	3.54	3.90	7.11
Liver weight (g)	307.7	317.4	344.2	364.2	272.5	317.0	290.6	288.8
Liver weight, relative (× 100)	2.42	2.31	2.88	3.52**	2.58	2.57	2.94	3.47*
Thyroid weight (g)	0.93	1.03	1.24	1.31	0.72	0.92	1.04*	1.00
Thyroid weight, relative (× 100 000)	7.36	7.63	10.43	12.67	6.79	7.49	10.46**	11.91**
Thyroid; follicular epithelial hypertrophy	0	0	0	4	0	0	2	3
Thyroid; follicular epithelial hyperplasia	0	0	0	1	0	0	0	1

From Auletta (1992)

* $p < 0.05$; ** $p < 0.01$

T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone.

The animals at the highest dose also had decreased serum alanine aminotransferase activity, increased alkaline phosphatase activity, increased cholesterol levels (also at the intermediate dose), and decreased albumin : globulin ratios (also at the intermediate dose), calcium (also at the intermediate dose), potassium and phosphorus concentrations. In females at 200 mg/kg bw per day, serum alanine aminotransferase activity was decreased, and alkaline phosphatase activity and cholesterol levels were increased. Thyroid function tests revealed decreased thyroxine concentrations in males at the intermediate and highest doses but no clear effects on triiodothyronine or thyroid-stimulating hormone. Abnormalities seen post mortem included increased liver weights in dogs at the highest dose and

increased thyroid weights in those at the intermediate and highest doses. Microscopic alterations attributed to treatment were limited to minimal to moderate hypertrophy and slight hyperplasia of the follicular epithelium of the thyroid in the dogs at the intermediate dose (hypertrophy in two females) and the highest dose (hypertrophy in four males and three females, and hyperplasia in one male and one female).

The NOAEL was 8 mg/kg bw per day, on the basis of increased thyroid weights and hypertrophy of the thyroid follicular epithelium at 40 mg/kg bw per day (Auletta, 1992).

In a short-term study of toxicity conducted in compliance with the test guidelines of the United States EPA, groups of four male and four female beagle dogs (aged approximately 7 months at the start of the study) were given gelatin capsules containing thiophanate-methyl (purity, 96.55%) at a dose of 0, 50, 200 or 800 mg/kg bw per day for 3 months, the last dose being lowered to 400 mg/kg bw per day on test day 50 because of severe toxicity. One male at the highest dose was sacrificed on day 41 because of severe toxicity; one male at 50 mg/kg bw per day died on day 36, but this death did not appear to be related to treatment. Dose-related clinical signs seen in animals at the highest dose and to a lesser extent at the intermediate dose included dehydration, thinness, and lethargy. Dose-related decreases in body weights and marked decreases in food consumption were seen at the intermediate and highest doses. There were no treatment-related ophthalmological findings. Slight anaemia, increased platelet counts and cholesterol levels, and decreases in serum alanine aminotransferase activity and albumin levels were seen at the intermediate and highest doses and increased activated partial thromboplastin time at the highest dose. Thyroid function tests revealed slightly decreased triiodothyronine levels in males at the highest dose and decreased triiodothyronine and thyroxine levels in females at the intermediate and highest doses; no clear effects on thyroid-stimulating hormone values were apparent. Urine analysis showed no treatment-related findings. At the intermediate and highest doses, the weights of the liver (in animals of each sex) and thyroid (males only) were increased. Gross examination post mortem revealed emaciation in one male at the intermediate dose and three at the highest dose. Dose-related histological alterations were seen in a number of organs. In thyroids, hypertrophy of the follicular epithelium was found in one, three and four males and one, two and four females at the lowest, intermediate and highest doses, respectively, with none in controls. The severity of the hypertrophy increased from minimal to marked with dose. Hyperplasia of the follicular epithelium was found in one male at the intermediate dose and in most animals at the highest dose. In two males at the highest dose in which both hypertrophy and hyperplasia were marked, a large decrease in the quantity of intrafollicular colloid was seen. In animals at the intermediate and highest doses, dose-related changes were found in the liver (reduction in vesiculation of the hepatocellular cytoplasm), gall-bladder (intracytoplasmic vacuoles), pancreas (atrophy of acinar cells owing to a decreased quantity of zymogen granules), spleen (lymphoid cell depletion), thymus (involution), prostate (atrophy), uterus (anestrus) and ovaries (inactive). No NOAEL was identified because of the presence of follicular cell hypertrophy in the thyroid of two dogs at the lowest dose (Auletta, 1991).

1.2 Genotoxicity

In a test for micronucleus formation performed in compliance with the test guidelines of the Organisation for Economic Co-operation and Development (OECD) and the United States EPA, groups of five male and five female B6D2F_1 mice were given thiophanate-methyl (purity, 97.28%) in 1% aqueus methylcellulose by oral gavage as single doses at 0, 500, 1000 or 2000 mg/kg bw, while a positive-control group was given carbendazim (purity, 98%) at a dose of 1000 mg/kg bw. Bone-marrow

smears were obtained from five males and five females in each group at 24 h and 48 h after dosing, with the exception that mice in the positive-control group were sampled at 24 h only. One smear from each animal was examined for the presence of micronuclei in 2000 immature erythrocytes. Doses were based on the results obtained in a preliminary study of toxicity in which plasma samples were taken to determine whether biologically significant concentrations of carbendazim can be expected after exposure to thiophanate-methyl in vivo (Table 3).

Table 3. Results of plasma analysis from a test for micronucleus formation in mice given single doses of thiophanate-methyl by gavage

Administered dose of thiophanate-methyl (mg/kg bw)	Sampling time (h)	Plasma concentration (ng/ml)[a]	
		Positive control (carbendazim)	Thiophanate-methyl
0	0	ND	ND
500	1	2033	5475
	3	411	2450
	6	559	1495
	12	28	55
	24	17	56
1000	1	3085	5370
	3	1483	3452
	6	651	691
	12	40	82
	24	< 5	< 5
2000	1	2776	7201
	3	1462	4311
	6	1578	4042
	12	567	2048
	24	47	76

From Proudlock (1999)

ND, not detected

[a] Mean values (from two males and two females).

Animals treated with thiophanate-methyl showed a small dose-related and statistically significant increase in the frequency of micronucleated immature erythrocytes at both sampling times, while a significant decrease in the proportion of immature erythrocytes was obtained in animals sampled at 24 h only. The positive control, carbendazim, produced large, significant increases in the frequency of micronucleated immature erythrocytes together with a significant decrease in the proportion of immature erythrocytes (Table 4).

Additional examinations (centromeric staining, size analysis of micronuclei) showed that carbendazim induced a high proportion (68%) of micronuclei containing centromers and a high proportion of large micronuclei (size, 40.1 units), while thiophanate-methyl produced an intermediate proportion of centromere-positive micronuclei (34%) and micronuclei of intermediate size (31.9 units). The clastogenic compound mitomycin C produced a low proportion of centromere-positive micronuclei (24%) and mainly small micronuclei (size, 31.9 units).

Table 4. Results of bone-marrow smear analysis from a test for micronucleus formation in mice given single doses of thiophanate-methyl by gavage

Sampling time (h)	Treatment	Dose (mg/kg bw)	Proportion of immature erythrocytes[a]	Micronuclei in immature erythrocytes[b]	Micronuclei in mature erythrocytes[c]
24	Vehicle control	0	45	1.3	0.3
	Thiophanate-methyl	500	46	4.2**	0.3
		1000	44	3.8**	0.9
		2000	41*	6.3**	1.2
	Positive control (carbendazim)	1000	38**	24.5**	2.2
48	Vehicle control	0	43	1.2	1.3
	Thiophanate-methyl	500	43	3.1**	0.3
		1000	45	5.0**	0.0
		2000	44	5.6**	1.6

From Proudlock (1999)

* $p < 0.01$; ** $p < 0.001$

[a] Proportion of immature erythrocytes [% immature erythrocytes / (immature erythrocytes + mature erythrocytes)]

[b] No. of micronucleated cells per 2000 immature erythrocytes examined.

[c] No. of micronucleated cells per 2000 mature erythrocytes examined.

1.3 Reproductive toxicity

(a) Multigeneration studies

In a dose range-finding study for a two-generation study of reproductive toxicity, groups of 7 male and 14 female Sprague-Dawley Crl:CD(SD)BR rats were fed diets containing thiophanate-methyl (purity, 95.93%) at a concentration of 0, 75, 200, 1200 or 6000 ppm from the start of the treatment until necropsy. After a pre-mating treatment period of 14 days, each male was mated with two females in the same dose group for a maximum of 3 weeks and the females were allowed to litter and to rear their offspring to weaning. Determinations for clinical signs, body weights, feed consumption, mating, fertility and litter size were performed during the study. Offspring were examined for sex ratio, viability, clinical signs and body-weight gain. The males were sacrificed shortly after the mating period and the dams and pups after weaning. Gross necropsy evaluations were performed on all adults and pups and liver and thyroid of parental animals were weighed. Dietary analyses revealed satisfactory test substance stability and concentration. The mean daily intakes at 75, 200, 1200 and 6000 ppm in the pre-mating period were 5.1–5.4, 13.6–14.5, 82.1–85.1 and 361.3–375.3 mg/kg bw per day in males and 6.3–6.5, 16.4–17.5, 101.9–102.5 and 474.2–480.8 mg/kg bw per day in females, respectively.

No mortalities or treatment-related clinical findings were observed during the study. Feed consumption at 6000 ppm was markedly reduced in males before mating, while in females it was slightly to moderately reduced during the entire study. Body-weight gain was slightly or markedly reduced in males at 1200 and 6000 ppm before and during mating and in females at 6000 ppm before and during mating and during gestation. Thyroid weights were moderately to markedly increased at 1200 and 6000 ppm in males and females, while liver weights were moderately increased in females at 1200 and 6000 ppm (Table 5). There were no treatment-related effects on fertility or reproductive performance and on the development of pups.

The NOAEL for parental toxicity was 200 ppm (equal to 13.6–14.5 mg/kg bw per day in males and 16.4–17.5 mg/kg bw per day in females, respectively), on the basis of reduced body-weight gain in males, increased thyroid weights in males and females and increased liver weights in females at 1200 ppm and above. The NOAEL for reproductive toxicity was 6000 ppm (equal to 361.3–375.3 mg/kg bw per day in males and 474.2–480.8 mg/kg bw per day in females, respectively), the highest dose tested (Müller, 1992).

Table 5. Selected findings in the parental generation of rats given diets containing thiophanate-methyl in a range-finding study of reproductive toxicity

Parameter	Dietary concentration (ppm)				
	0	75	200	1200	6000
Males					
Feed consumption (g/day), pre-mating, days 11–15	33.4	32.0	32.6	31.8	26.6**
Body-weight gain (g), pre-mating, days 1–15	52.6	54.8	60.5	47.5	28.8*
Body weight (g), termination	524.4	522.0	516.1	508.2	475.8*
Thyroid weight (mg)	25.6	29.0	24.6	31.4	49.3*
Liver weight (g)	22.3	24.6	22.1	24.3	24.0
Females					
Feed consumption (g/day), pre-mating, days 11–15	22.3	22.7	21.9	22.0	19.9*
Feed consumption (g/day), gestation, days 0–20	26.4	26.9	27.6	27.3	23.0**
Feed consumption (g/day), lactation, days 0–21	55.8	47.3	57.2	59.5	46.5
Body-weight gain (g), pre-mating, days 1–15	30.2	31.5	30.0	29.5	18.1**
Body-weight gain (g), gestation, days 0–20	153.7	156.9	160.5	161.3	131.7*
Body weight (g), termination	325.2	332.5	337.2	335.9	312.2
Thyroid weight (mg)	20.2	23.2	21.8	29.8*	48.4*
Liver weight (g)	17.5	17.1	19.0	20.9*	20.9*

From Müller (1992)

* $p \leq 0.05$; ** $p \leq 0.01$

(b) Developmental toxicity

Mice

In a study of prenatal developmental toxicity, groups of 20 pregnant ICR mice were given thiophanate-methyl (purity, not reported) in 5% gum arabic aqueous solution by gavage at a dose of 0, 40, 200, 500 or 1000 mg/kg bw per day on days 1–15 of gestation (the day after overnight mating being considered to be day 1). The mice were observed for clinical signs and were weighed on days 1, 15 and 19. The animals were sacrificed on day 19 of gestation, and the numbers of implantation sites and live and dead fetuses were counted. The live fetuses were examined for sex, body weight, and gross external, visceral, and skeletal alterations.

No signs of toxicity were observed in the dams, throughout the study period. There were no significant differences in the number of implantation sites, fetal body weights or the incidence of

malformations between the control and the dosed groups. At 1000 mg/kg bw per day, the number of live fetuses was significantly decreased compared with the controls (Table 6).

Table 6. Selected maternal and reproductive findings in a study of prenatal developmental toxicity in mice given thiophanate-methyl by gavage

Parameter	Dose (mg/kg bw per day)				
	0	40	200	500	1000
Body weight (g), day 19	56.5	54.5	51.8	52.4	52.8
Total No. of implantations	244	225	221	233	225
Mean No. of implantations	12.20	11.25	11.05	11.65	11.25
Total No. of live fetuses	218	204	202	212	195*
Mean No. of live fetuses	10.90	10.20	10.10	10.60	9.70*
Mean fetal weight, live fetuses (g)	1.13	1.12	1.18	1.14	1.10
Malformed live fetuses (No. / %)[a]	2/0.77	1/0.44	2/0.90	1/0.43	2/0.89
Dead fetuses; immature (No. / %)	17/6.97	9/4.00	10/4.53	13/6.45	12/5.33
Dead fetuses; resorbed (No. / %)	9/3.69	12/5.33	9/4.07	6/2.57	18/8.00

From Noguchi & Hashimoto (1970)

* $p \leq 0.05$

[a] Fetuses with cleft palate.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 500 mg/kg bw per day, on the basis of decreased number of live fetuses at 1000 mg/kg bw per day (Noguchi & Hashimoto, 1970).

Rabbits

In a dose range-finding study for a prenatal developmental study of toxicity, groups of 6 presumed pregnant New Zealand white [Hra:(NZW)SPF] rabbits were given technical-grade thiophanate-methyl at a dose of 0, 5, 10, 20, 40 or 80 mg/kg bw per day in 1% methylcellulose by gavage on days 6 to 28 of gestation. The rabbits were sacrificed on day 29 of gestation. The does were observed for viability, clinical signs, body weight, food consumption and the number of corpora lutea, and the thoracic, abdominal, and pelvic viscera were examined. Their uteri were excised and examined for the number and distribution of implantation sites, live and dead fetuses and early and late resorptions. The fetuses were examined for sex and gross external alterations, and the body weights were recorded. Fetuses from the control, lowest- and highest-dose groups were also examined for visceral and skeletal alterations.

At 80 mg/kg bw per day, there were two abortions on days 23 and 24, respectively, which were considered to be treatment-related. Regarding clinical observations, faecal output, feed consumption and body-weight gain were reduced over the entire treatment period at 40 mg/kg bw per day and above, while at 20 mg/kg bw per day, feed consumption and body-weight gain were reduced only on days 6 to 9 of gestation (Table 7). At 80 mg/kg bw per day, there was an increase in the number of early resorptions and a concomitant decrease in litter size. In the fetuses examined for visceral and skeletal alterations, the number of thoracic rib pairs was increased at 80 mg/kg bw per day, with associated increases and decreases in the averages for thoracic and lumbar vertebrae, respectively (York, 1997a).

Table 7. Selected maternal and reproductive findings in a range-finding study of prenatal developmental toxicity in rabbits given thiophanate-methyl by gavage

Parameter	Dose (mg/kg bw per day)					
	0	5	10	20	40	80
Body-weight gain (kg), days 6–9	0.05	0.07	0.08	–0.04	–0.18	–0.24
Body-weight gain (kg), days 6–29	0.43	0.26	0.26	0.30	0.12	0.03
Feed consumption (g/day), days 6–9	190.1	172.9	185.1	121.1	65.5	11.1
Feed consumption (g/day), days 6–29	167.7	144.4	144.6	137.6	101.8	81.2
No. of dams pregnant/with live fetuses	6/6	6/5	5/5	5/5	6/6	6/3
No. of dams aborted/found dead	0/0	0/1	0/0	0/0	0/0	2/0
Mean No. of corpora lutea/implantations	9.7/8.8	7.8/7.4	9.2/8.8	9.6/9.0	9.0/8.3	9.8/9.0
Mean litter size	8.8	7.2	8.2	8.6	8.3	5.2
No. of live/dead fetuses	53/0	36/0	41/0	43/0	50/0	21/0
No./mean No. of early resorptions	0	1/0.2	2/0.4	1/0.2	0	13/3.2
No./mean No. of late resorptions	0	0	1/0.2	1/0.2	0	2/0.5
No./% of does with any resorption	0	1/20	2/40	2/40	0	3/75
Mean % resorbed conceptuses per litter	0	3.3	5.5	4.7	0	10.3
Mean live fetal body weights (g)	39.61	40.91	41.37	40.19	40.27	40.02
Litters/fetuses with any alterations	4/7	3/4	1/1	0	0	1/1
% Fetuses with any alteration per litter	12.7	8.1	1.5	0	0	6.7
Mean No. of ribs (pairs)	12.45	12.24	NE	NE	NE	12.87
Mean No. of cervical vertebrae	7.00	7.00	NE	NE	NE	7.00
Mean No. of thoracic vertebrae	12.48	12.34	NE	NE	NE	12.90
Mean No. of lumbar vertebrae	6.48	6.66	NE	NE	NE	6.10
Mean No. of sacral vertebrae	3.00	3.00	NE	NE	NE	3.00
Mean No. of caudal vertebrae	16.75	16.86	NE	NE	NE	17.10

From York (1997a)

NE, not examined.

In a study of prenatal developmental toxicity, groups of 20 naturally bred New Zealand white [Hra:(NZW)SPF] rabbits were given technical-grade thiophanate-methyl (purity, 97.28–97.57%) at a dose of 0, 5, 10, 20 or 40 mg/kg bw per day in 1% methylcellulose by gavage on days 6–28 of gestation, the day of mating being considered to be day 0. The rabbits were sacrificed on day 29 of gestation. The does were observed for viability, clinical signs, body weight, food consumption, and the number of corpora lutea and the thoracic, abdominal, and pelvic viscera were examined. Their uteri were excised and examined for the number and distribution of implantation sites, live and dead fetuses and early and late resorptions. The fetuses were observed for sex, body weight and gross external, visceral, brain and skeletal alterations.

At a dose of 20 mg/kg bw per day, there was a transient but significant reduction in maternal body-weight gain and statistically significant reduced absolute and relative feed consumption. At a dose of 40 mg/kg bw per day, faecal output was reduced, in conjunction with significantly reduced maternal body-weight gain and absolute and relative feed consumption; however, body-weight gain and food consumption recovered after the initial week of dosing. It is likely that the observed reduction in feed consumption during this period contributed to the observed decreases in body weight, body-weight gain and faecal output. There appeared to be an increased incidence of resorptions in rabbits treated with 40 mg/kg bw per day, the numbers of litters with ≥ 20% resorptions per litter being 2 of 20 in the controls, 2 of 17 at 5 mg/kg bw per day, 2 of 18 at 10 mg/kg bw per day, 0 of 17 at 20 mg/kg bw per day, and 5 of 20 at 40 mg/kg bw per day. The historical control range for dead or resorbed conceptuses per litter was 0–18.3%. In the fetuses, the dose of 40 mg/kg bw per day caused a significant increase in the average for thoracic ribs (supernumerary ribs), with associated significant increases and decreases in the averages for thoracic and lumbar vertebrae, respectively. In addition, fetal body weights were slightly reduced (about 9%; not statistically significant) at the dose of 40 mg/kg bw per day (Table 8).

The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced feed consumption and reduced body-weight gain at 20 mg/kg bw per day and above. The NOAEL for developmental effects was 20 mg/kg bw per day, on the basis of increased incidence of supernumerary thoracic ribs and decreased fetal weights at 40 mg/kg bw per day, the highest dose tested. Thiophanate-methyl was not selectively toxic to embryo or fetal viability, growth or morphology and was not teratogenic (York, 1997b).

Table 8. Selected maternal and reproductive findings in a study of prenatal developmental toxicity in rabbits given thiophanate-methyl by gavage

Parameter	Dose (mg/kg bw per day)				
	0	5	10	20	40
Body-weight gain (kg), days 6–9	0.08	0.07	0.09	0.06	–0.11**
Body-weight gain (kg), days 12–15	0.09	0.11	0.08	0.04*	0.03**
Body-weight gain (kg), days 6–29	0.41	0.44	0.44	0.36[c]	0.10**
Feed consumption (g/day), days 6–9	180.7	175.0	182.4	152.3	58.8**
Feed consumption (g/day), days 6–29	159.0	160.6	160.9	140.7*	89.3**
No. of dams pregnant/with live fetuses	20/19[a]	17/17	19/18[b]	17/17	20/19[a]
Mean No. of corpora lutea/implantations	10.3/9.3	9.4/8.9	9.9/9.7	8.6/7.6	9.9/9.2

Mean litter size	8.8	8.4	9.1	7.4	8.4
No. of live/dead fetuses	168/0	141/2	164/0	125/0	160/0
No./mean No. of early resorptions	7/0.4	5/0.3	5/0.3	3/0.2	7/0.4
No./mean No. of late resorptions	2/0.1	3/0.2	5/0.3	1/0.0	7/0.4
No./% of does with any resorption	7/36.8	7/41.2	6/33.3	4/23.5	9/47.4
No. of does with ≥ 20% resorptions	2	2	2	0	5
No. of litters/fetuses evaluated	19/168	17/143	18/164	16/115[c]	19/160
Mean live fetal body weights (g)	44.78	43.05	42.68	45.56	40.64
Litters/fetuses with any alterations	11/14	12/23**	8/11	13/21**	10/16
% Fetuses with any alteration per litter	8.9	15.4	7.5	18.6*	10.0
Mean No. of ribs (pairs)[a]	12.45	12.44	12.45	12.58	12.85**
Mean No. of cervical vertebrae	7.00	7.00	7.00	7.00	7.00
Mean No. of thoracic vertebrae[b]	12.50	12.52	12.53	12.68	12.89**
Mean No. of lumbar vertebrae[c]	6.48	6.47	6.46	6.32	6.09**
Mean No. of sacral vertebrae	3.00	3.00	3.00	3.00	3.00
Mean No. of caudal vertebrae	16.96	17.02	17.04	16.98	17.06

From York (1997b)

* $p \leq 0.05$; ** $p \leq 0.01$

[a] Historical control range: ribs: 12.34–12.67; thoracic vertebrae: 12.38–12.70; lumbar vertebrae: 6.30–6.61

[a] Excludes values for does that had litters consisting of all early resorptions.

[b] Excludes values for doe which delivered on day 12 of presumed gestation (mating date incorrectly identified).

[c] Excludes values for doe which prematurely delivered on day 29 of gestation.

1.4 Special studies: neurotoxicity

In a study of acute neurotoxicity that complied with the test guidelines of the United States EPA and OECD, groups of 10 male and 10 female Crl:CD(SD)IGS BR VAF Plus rats were given thiophanate-methyl (purity, 99.7%) at a dose of 0, 500, 1000 or 2000 mg/kg bw as a suspension in aqueous 0.5% (w/v) methylcellulose by gavage once on day 1 of the study . In the extension part of the study, which was conducted to evaluate additional dosages for several parameters affected in the main study, groups of 10 male and 10 female rats were given thiophanate-methyl at a dose of 0, 50, 125, 500 or 2000 mg/kg bw by gavage on day 1 of the study. The dosage volume was 10 ml/kg in both study phases.

In the main study, viabilities, clinical observations, body weights, feed consumption values, functional observational battery (FOB) evaluations (which included detailed clinical observations) and motor activity evaluations were recorded. Rats were sacrificed on day 15, administered a

combination of heparin and an anaesthetic and perfused in situ with neutral buffered 10% formalin. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Brain weights were recorded for all rats after additional fixation of the tissue. Five rats of each sex per group were selected for neurohistological examination; and the tissues from rats selected in the control groups and group at the highest dose were processed for histological examination. In the study extension, viabilities, clinical observations, body weights, feed consumption values, two components of the FOB (behaviour in the home cage and measurement of landing foot splay) and motor activity evaluations (female rats only) were evaluated. These rats were sacrificed on day 3 of the study, and carcasses were discarded without further evaluation.

All rats survived until scheduled necropsy on day 15 (main study) or day 3 (extension study). None of the clinical signs observed in these rats were considered to be related to the test substance. Body weights and feed consumption values were unaffected at a dose of 50 or 125 mg/kg bw in the extension study. Transient reductions in body-weight gains or body-weight losses along with corresponding reductions in absolute (g/day) and relative (g/kg per day) feed consumption occurred on the day after dosage in both sexes given thiophanate-methyl at 500 mg/kg bw and higher in the main study, the extension study or both. These changes were followed by increases in body-weight gain on the subsequent day.

In the FOB conducted 2 h after dosing in the main study, both male and female rats given thiophanate-methyl at 500, 1000 or 2000 mg/kg bw had significantly reduced values for landing foot splay (Table 9). The numbers of male rats in these groups that appeared to be sleeping when examined in the home cage also were significantly reduced, and the numbers showing other normal patterns (e.g. appeared awake and immobile or showed normal movement) were increased when compared with the control group. Female rats at 500 mg/kg bw and higher also had significantly reduced motor activity during the test that followed the FOB examination on the day of dosing. There were neither statistically significant nor exceptional differences among the groups in the measures of the functional observational battery or in the motor activity test on the day before dosage and 7 days and 14 days after dosing. No gross lesions were observed at necropsy. Brain weights and the ratio of brain weights to terminal body weights were comparable among the groups. No lesions related to the test substance were observed in the microscopic examination of the neural and muscle tissues.

The extension study evaluated home cage behaviour and landing foot splay in both sexes and motor activity in females dosing dosage and 2 h after dosing. Landing foot-splay values were significantly reduced in both sexes at 50 mg/kg bw and higher (Table 9). The variations in normal home-cage behaviour and reduced motor activity observed in the main study were not observed at 50 and 125 mg/kg bw and were not replicated at 500 and 2000 mg/kg bw in the study extension. These changes were considered to be incidental events because they were noted only in one sex in the main study and not reproduced in the extension study. This conclusion is also supported by the evaluations in the short-term study of neurotoxicity in which there were no adverse changes in the FOB, including the home-cage observation and motor activity tests at the highest dietary concentration of 2500 ppm at 2, 4, 8 and 13 weeks of exposure.

The toxicological significance of the transient decreases in landing foot splay observed in this study is questionable and considered inappropriate for setting a NOAEL. The biological significance of decreased landing foot splay in the absence of apparent changes in gait, posture or other behavioural responses such as air righting has not been specified in previous research. In addition, the variability observed within the pairs of measurements obtained from individual rats as well as variability among the averages for rats in the control group in this study and across other studies were comparable to the differences between the control groups and the groups given thiophanate-methyl. This conclusion is also supported by the evaluations in the short-term study of neurotoxicity in which there were no

adverse changes in the FOB, including the landing foot-splay measurements, at the highest dietary concentration of 2500 ppm (equal to 149.6 mg/kg bw per day in males and 166.3 mg/kg bw per day in females) at 2, 4, 8 and 13 weeks of exposure.

Table 9. Landing foot splay (cm) in rats given a single dose of thiophanate-methyl by gavage in a study of acute neurotoxicity

Sex	Study[a]	Time-point	Dose (mg/kg bw)					
			0	50	125	500	1000	2000
Males	Main study	Before dosing	7.0	NE	NE	7.1	6.6	7.4
		2 h after dosing	6.6	NE	NE	5.2*	4.2**	4.7**
		7 days after dosing	6.5	NE	NE	6.9	6.6	7.0
		14 days after dosing	6.4	NE	NE	6.8	6.1	6.4
	Study extension	Before dosing	8.4	8.9	8.2	7.6	NE	8.2
		2 h after dosing	8.9	7.0**	6.5**	5.2**	NE	6.1**
Females	Main study	Before dosing	6.2	NE	NE	6.5	6.4	7.2
		2 h after dosing	5.6	NE	NE	3.9**	4.2**	4.4**
		7 days after dosing	5.5	NE	NE	5.1	5.6	5.8
		14 days after dosing	5.3	NE	NE	4.8	5.5	5.9
	Study extension	Before dosing	8.4	8.0	8.3	7.4	NE	7.9
		2 h after dosing	8.4	6.8*	6.0**	6.0**	NE	5.6**

From Foss (2005a)

* $p \leq 0.05$; ** $p \leq 0.01$

NE, not examined.

[a] Data for historical controls: males 6.5 ± 1.7 cm and 8.7 ± 1.2 cm; females, 6.3 ± 1.2 cm and 5.7 ± 1.7 cm.

The NOAEL for general toxicity was 125 mg/kg bw on the basis of transient reductions in body-weight gains (including body-weight losses) and feed consumption at 500 mg/kg bw and above. The NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested. The toxicological significance of the transient decreases in landing foot splay observed at all doses was questionable and was considered to be inappropriate for identifying a NOAEL for neurotoxicity (Foss, 2005a).

In a short-term study of neurotoxicity conducted in compliance with the test guidelines of the United States EPA and OECD, groups of 10 male and 10 female Crl:CD(SD)IGS BR VAF Plus rats were fed diets containing thiophanate-methyl (purity, 99.7%) at a concentration of 0, 100, 500 or 2500 ppm for 13 weeks (91 days). Viability, clinical observations, body-weights, feed consumption, functional observational battery (FOB) evaluations (which included detailed clinical observations) and motor activity evaluations were recorded. At termination rats were administered a combination of heparin and an anaesthetic and perfused in situ with neutral buffered 10% formalin. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Liver and individual kidney weights were recorded at necropsy, and brain weights were recorded after additional fixation of the tissue. Five rats of each sex per group were selected for neurohistological examination; and the tissues from rats selected in the control group and the group receiving the highest dietary concentration were evaluated.

Table 10. Selected findings from a short-term study of neurotoxicity in rats given diets containing thiophanate-methyl for 13 weeks

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	100	500	2500	0	100	500	2500
Body weight (g), day 91	540.6	532.8	544.5	519.9	309.9	290.1	296.3	280.2
Body-weight gain (g), days 1–91	329.1	323.6	335.0	309.7	140.0	121.7*	127.3	110.6**
Feed consumption (g/day)	24.7	25.1	24.9	23.8	18.7	16.8**	17.6	15.9**
Forelimb grip, maximum (g), week 2	284.5	335.5	335.5	340.0	291.5	201.0**	264.0	222.5*
Forelimb grip, average (g), week 2	257.2	295.8	303.2	289.2	259.8	177.8**	235.0	204.5*
Hindlimb grip, maximum (g) week 2	296.5	338.5	366.5	291.5	279.5	202.0**	206.5*	213.0*
Hindlimb grip, average (g), week 2	259.2	315.5	325.2	262.0	236.8	184.5*	187.8*	187.2*
Liver								
Absolute weight (g)	22.95	20.75	23.91	27.66*	12.95	11.80	12.88	13.92
Relative weight (g/100 g bw)	4.24	3.89	4.37	5.32**	4.19	4.06	4.34	4.97**
Thyroid								
Absolute weight (mg)	29	30	34	57**	28	21	26	35
Relative weight (mg/100 g bw)	5.45	5.72	6.25	10.97**	8.87	7.03	8.84	12.45**

From Foss (2005b)

* $p \leq 0.05$; ** $p \leq 0.01$

Mean intakes of test substance were 6.2, 30.3 and 149.6 mg/kg bw per day for males and 6.8, 34.9 and 166.3 mg/kg bw per day for females at 100, 500 and 2500 ppm, respectively. None of the clinical signs observed in the daily examinations were considered to be related to the test substance. Body weights, body-weight changes and absolute and relative feed consumption values were significantly decreased at 2500 ppm in females, while these values were unaffected in males (Table 10). Other than the body weights in the female rats, none of the parameters evaluated in the FOB and motor activity test sessions were affected by the dietary concentrations of thiophanate-methyl administered in this study. The decreases in the forelimb and hindlimb grip in females at 100 ppm and higher during week 2 of exposure were considered to be incidental events unrelated to the test substance, because the differences were not dose-dependent and there were no effects on other FOB parameters that might be affected by muscle weakness. There were no gross lesions in either sex, but the absolute weights of the liver and thyroid as well as ratios of these weights to terminal body weight and to brain weight were significantly increased at 2500 ppm in male rats; the ratios of these weights to terminal body weight were also significantly increased in female rats at this concentration. No microscopic lesions were observed in the neural or muscle tissues of the rats in the control group or at 2500 ppm.

The NOAEL for general toxicity was 500 ppm (equal to 30.3 and 34.9 mg/kg bw per day in males and females, respectively) on the basis of decreased body weights and feed consumption in females and increased liver and thyroid weights in both sexes at 2500 ppm. The NOAEL for neurotoxicity was 2500 ppm (equal to 149.6 and 166.3 mg/kg bw per day in males and females, respectively), the highest dose tested (Foss, 2005b).

Comments

Toxicological data

Thiophanate-methyl has low acute toxicity: the oral median lethal dose (LD_{50}) was 6640–7500 mg/kg bw in rats and 3400–3514 mg/kg bw in mice. The clinical signs of toxicity after single high (near-lethal) doses included whole body tremors at 1–2 h after dosing, which progressed to tonic and clonic convulsions.

In a 1-year study of toxicity in dogs given capsules containing thiophanate-methyl, slight tremors were observed in all eight animals approximately 2–4 h after administration of the highest dose of 200 mg/kg bw per day on one to five occasions during the initial 17 days of the study. One dog exhibited severe tremors that progressed to tonic convulsions on three occasions. The NOAEL was 8 mg/kg bw per day on the basis of increased thyroid weights and hypertrophy of the thyroid follicular epithelium at 40 mg/kg bw per day and above.

In a 3-month study of toxicity in dogs given capsules containing thiophanate-methyl at doses of up to 800 mg/kg bw per day, dose-related clinical signs including dehydration, thinness and lethargy were seen in animals at the highest dose and to a lesser extent at the intermediate dose (200 mg/kg bw per day). No tremors were seen up to the highest dose tested. A NOAEL could not be identified in this study because of the presence of follicular-cell hypertrophy in the thyroid of two dogs at 50 mg/kg bw per day, the lowest dose tested.

Thiophanate-methyl has been adequately tested in a range of assays for genotoxicity. Thiophanate-methyl does not cause gene mutations or structural chromosomal aberrations; however, it causes changes in chromosome number (aneuploidy) both in vitro and in vivo. Induction of micronucleus formation in mice was seen after single highest doses (500 mg/kg bw and above), but the response was weak when compared with that for the main metabolite of thiophanate-methyl, carbendazim, which is considered to be responsible for the observed effect. The mechanism by which aneuploidy is induced by carbendazim is clearly understood and there is a clear threshold for this effect.

The Meeting concluded that the genotoxic effect of thiophanate-methyl is a threshold phenomenon and is related to the production of carbendazim.

On the basis of evaluations from previous Meetings, there was no adverse effect on fertility and reproductive performance in a recent two-generation study of reproduction toxicity using doses of up to 2000 ppm, equal to 147.1 and 164.3 mg/kg bw per day in males and females, respectively.

The developmental toxicity potential of thiophanate-methyl had been investigated in mice, rats and rabbits. From evaluations made at previous Meetings, the NOAEL for developmental toxicity in mice was 500 mg/kg bw per day, on the basis of decreased number of live fetuses at 1000 mg/kg bw per day, while no maternal toxicity was observed at this dose. In rats, there was no evidence of developmental toxicity at doses of up to 1000 mg/kg bw per day, but maternal toxicity (reduced body-weight gain) was seen at this dose.

In a study of prenatal developmental toxicity in rabbits, which had not been evaluated previously, the NOAEL for developmental effects was 20 mg/kg bw per day on the basis of increased incidence of supernumerary thoracic ribs and decreased fetal weights at 40 mg/kg bw per day, the highest dose tested. The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced feed consumption and reduced body-weight gain at 20 mg/kg bw per day and above.

Thiophanate-methyl was not selectively toxic to embryo or fetal development in rats and rabbits and was not teratogenic.

In a study of acute neurotoxicity in rats, the NOAEL for general toxicity was 125 mg/kg bw on the basis of transient reductions in body-weight gains (including body-weight losses) and feed consumption at 500 mg/kg bw and above. The NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested.

In a short-term study of neurotoxicity in rats, the NOAEL for general toxicity was 500 ppm (equal to 30.3 and 34.9 mg/kg bw per day in males and females, respectively) on the basis of decreased body weights and feed consumption in females and increased liver and thyroid weights in both sexes at 2500 ppm. No neurohistological changes were seen at 2500 ppm. The NOAEL for neurotoxicity was 2500 ppm (equal to 149.6 and 166.3 mg/kg bw per day in males and females, respectively), the highest dose tested.

Toxicological evaluation

The Meeting considered whether the establishment of an ARfD was necessary. The initial, transient clinical signs (tremors) that were seen in a 1-year study in dogs given thiophanate-methyl at a dose of 200 mg/kg bw per day were not observed in a 3-month study in dogs given thiophanate-methyl at doses of up to 800 mg/kg bw per day. Therefore the Joint Meeting considered that the tremors were not attributable to a toxicological effect of the test substance.

The developmental effects that had been observed in rabbits at 40 mg/kg bw per day were not considered to be elicited by a single exposure.

The Meeting concluded that it was not necessary to establish an ARfD for thiophanate-methyl in view of its low acute toxicity, the absence of relevant developmental toxicity that could be a consequence of acute exposure, the absence of relevant findings in a study of acute neurotoxicity, and the absence of any other toxicological effect that would be likely to be elicited by a single dose.

The Meeting noted that the use of thiophanate-methyl on crops may give rise to residues of carbendazim, although thiophanate-methyl can also be detected as part of the residue to which consumers of treated produce are exposed. Since the toxicity of thiophanate-methyl is qualitatively and quantitatively (when corrected for relative molecular mass) different from that of carbendazim, and since the ARfD for carbendazim is lower than that which would be derived from data on thiophanate-methyl, the Joint Meeting concluded that the intake of residues in food should initially be compared with the ARfD for carbendazim. If further refinement of the risk assessment is necessary, the different components of the residue (carbendazim and thiophanate-methyl) could be considered separately.

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 exposures

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York, R.G. (1997b) Oral (stomach tube) developmental toxicity study of thiophanate-methyl in rabbits. Unpublished report No. RD-9770, project No. 914-002 from Argus Research Laboratories, Inc., Horsham, Pennsylvania, USA. Submitted to WHO by Nippon Soda Co., Ltd., Tokyo, Japan.

ANNEX 1

Reports and other documents resulting from previous Joint Meetings Of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and WHO Expert Groups 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.

4. Evaluation of the toxicity of pesticide residues in food. FAO Meeting Report, No. PL/1965/10/1; WHO/Food Add./27.65, 1965.

5. Evaluation of the hazards to consumers resulting from the use of fumigants in the protection of food. FAO Meeting Report, No. PL/1965/10/2; WHO/Food Add./28.65, 1965.

6. Pesticide residues in food. Joint report of the FAO Working Party on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 73; WHO Technical Report Series, No. 370, 1967.

7. Evaluation of some pesticide residues in food. FAO/PL:CP/15; WHO/Food Add./67.32, 1967.

8. Pesticide residues. Report of the 1967 Joint Meeting of the FAO Working Party and the WHO Expert Committee. FAO Meeting Report, No. PL:1967/M/11; WHO Technical Report Series, No. 391, 1968.

9. 1967 Evaluations of some pesticide residues in food. FAO/PL:1967/M/11/1; WHO/Food Add./68.30, 1968.

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11. 1968 Evaluations of some pesticide residues in food. FAO/PL:1968/M/9/1; WHO/Food Add./69.35, 1969.

12. Pesticide residues in food. Report of the 1969 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Group on Pesticide Residues. FAO Agricultural Studies, No. 84; WHO Technical Report Series, No. 458, 1970.

13. 1969 Evaluations of some pesticide residues in food. FAO/PL:1969/M/17/1; WHO/Food Add./70.38, 1970.

14. Pesticide residues in food. Report of the 1970 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 87; WHO Technical Report Series, No. 4574, 1971.

15. 1970 Evaluations of some pesticide residues in food. AGP:1970/M/12/1; WHO/Food Add./71.42, 1971.

16. Pesticide residues in food. Report of the 1971 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 88; WHO Technical Report Series, No. 502, 1972.

17. 1971 Evaluations of some pesticide residues in food. AGP:1971/M/9/1; WHO Pesticide Residue Series, No. 1, 1972.

18. Pesticide residues in food. Report of the 1972 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 90; WHO Technical Report Series, No. 525, 1973.

19. 1972 Evaluations of some pesticide residues in food. AGP:1972/M/9/1; WHO Pesticide Residue Series, No. 2, 1973.

20. Pesticide residues in food. Report of the 1973 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 92; WHO Technical Report Series, No. 545, 1974.

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This volume contains toxicological monographs that were prepared by the 2006 Joint FAO/WHO Meeting on Pesticide Residues (JMPR), which met in Rome on 3–12 October, 2006.

The monographs in this volume summarize the safety data on 13 pesticides that could leave residues in food commodities or drinking water. These pesticides are bifenazate, boscalid, cyfluthrin/beta-cyfluthrin, cypermethrins (including alpha- and zetacypermethrin, cyromazine, diazinon, haloxyfop (haloxyfop-R and haloxyfop-R methylester), pyrimiphos-methyl, quinoxyfen, themephos, thiabendazole, thiacloprid and thiophanate-methyl. 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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