Pesticide residues in food – 2008

Joint FAO/WHO Meeting on Pesticide Residues

EVALUATIONS 2008

Part II — Toxicological

Food and Agriculture Organization of the United Nations

World Health Organization

Pesticide residues in food — 2008

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
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Rome, 9 - 18 September 2008

PARTICIPANTS

FAO Panel of Experts on Pesticide Residues in Food and the Environment

Professor Eloisa Dutra Caldas, University of Brasilia, College of Health Sciences, Pharmaceutical Sciences Department, Brasília/DF, Brazil

Mr Stephen Funk, Health Effects Division, United States Environmental Protection Agency, Washington, USA (*FAO Chairman*)

Mr Denis J. Hamilton, Principal Scientific Officer, Biosecurity Queensland, Department of Primary Industries and Fisheries, Brisbane, Australia

Mr David Lunn, Senior Programme Manager (Residues–Plants), Export Standards Group, New Zealand Food Safety Authority, Wellington, New Zealand (*FAO Rapporteur*)

Dr Dugald MacLachlan, Australian Quarantine and Inspection Service, Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, Australia

Dr Yukiko Yamada, Deputy Director-General, Food Safety and Consumer Affairs Bureau Ministry of Agriculture, Forestry and Fisheries, Chiyoda-ku, Tokyo, Japan

Toxicological Core Assessment Group

Professor Alan R. Boobis, Experimental Medicine & Toxicology, Division of Investigative Science, Faculty of Medicine, Imperial College London, London, United Kingdom

Dr Les Davies, Chemical Review, Australian Pesticides & Veterinary Medicines Authority, Kingston, Australia

Dr Vicki L. Dellarco, Health Effects Division, Office of Pesticide Programs, US Environmental Protection Agency, Washington, DC, USA (*WHO Rapporteur*)

Professor Angelo Moretto, Department of Environmental and Occupational Health, University of Milan, International Centre for Pesticides and Health Risk Prevention (ICPS), Milano, Italy (*WHO Chairman*)

Dr David Ray, Biomedical Sciences, University of Nottingham, Queens Medical Centre, Nottingham, United Kingdom

Dr Roland Solecki, Chemical Safety Division, Management and Overall Assessment, Federal Institute for Risk Assessment, Berlin, Germany

Dr Maria Tasheva, Consultant, National Service for Plant Protection, Ministry of Agriculture and Food, Sofia, Bulgaria

Secretariat

Mr Árpád Ambrus, Hungarian Food Safety Office, Budapest, Hungary (*FAO Temporary Adviser*)

Ms Catherine Adcock, Toxicological Evaluation Section 2, Health Effects Division II, Health Evaluation Directorate, Pest Management Regulatory Agency, Canada (*WHO Temporary Adviser*)

Mr Kevin Bodnaruk, West Pymble, NSW, Australia (*FAO Editor*)

Professor Zongmao Chen, Chairman of Codex Committee on Pesticide Residues, Academician, Chinese Academy of Engineering, Chinese Academy of Agricultural Sciences, P.R. China (*CCPR Chairman*)

Dr Myoengsin Choi, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland (*WHO Staff Member*)

Dr Ian Dewhurst, Pesticides Safety Directorate, York, United Kingdom (*WHO Temporary Adviser*)

Dr Ronald D. Eichner, Wanniassa ACT, Australia (*FAO Temporary Adviser*)

Dr Yibing He, Department of Science and Education, Ministry of Agriculture, Beijing P.R. China (*FAO Temporary Adviser*)

Mr George Herndon, Deputy Director, Health Effects Division, Office of Pesticide Programs, US Environmental Protection Agency, Washington, DC, USA (*FAO Temporary Adviser*)

Mr Makoto Irie, Plant Product Safety Division, Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries, Tokyo, Japan (*FAO Temporary Adviser*)

Dr D. Kanungo, Additional DG, Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi, India (*WHO Temporary Adviser*)

Dr Katerina Mastovska, Eastern Regional Research Center (ERRC), Agricultural Research Service (ARS), United States Department of Agriculture, Wyndmoor, PA,USA (*FAO Temporary Adviser*)

Dr Jeronimas Maskeliunas, Food Standards Officer, Joint FAO/WHO Food Standards Programme, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy (*Codex Secretariat*)

Dr Heidi Mattock, Tignieu-Jameyzieu, France (*WHO Editor*)

Dr Douglas B. McGregor, Toxicity Evaluation Consultants, Aberdour, Scotland (*WHO Temporary Adviser*)

Dr Francesca Metruccio, Department of Environmental and Occupational Health, University of Milan, International Centre for Pesticides and Health Risk Prevention (ICPS), Milan, Italy (*WHO Temporary Adviser*)

Dr Rudolf Pfeil, Toxicology of Pesticides and Biocides, Federal Institute for Risk Assessment, Berlin, Germany (*WHO Temporary Adviser*)

Dr Prakashchandra V. Shah, US Environmental Protection Agency (EPA), Washington DC, USA (*WHO Temporary Adviser*)

Mr Christian Sieke, Federal Institute for Risk Assessment, Berlin, Germany (*FAO Temporary Adviser*)

Ms Yong Zhen Yang, FAO Joint Secretary, Plant Protection Service (AGPP), Food and Agriculture Organization of the United Nations (FAO), Rome, Italy (*FAO Joint Secretary*)

Dr. Midori Yoshida, Section Chief, Division of Pathology, Biological Safety Research Center, National Institute of Health Sciences, Ministry of Health, Labour and Welfare, Tokyo, Japan (*WHO Temporary Adviser*)

Dr Angelika Tritscher, WHO Joint Secretary, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland (*WHO Joint Secretary; unable to attend*)

Dr Gerrit Wolterink, Centre for Substances & Integrated Risk Assessment, National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands (*WHO Temporary Adviser*)

Dr Jürg Zarn, Swiss Federal Office of Public Health, Food Toxicology Section, Zurich, Switzerland (*WHO Temporary Adviser*)

Abbreviations used

3-MC	3-methylcholanthrene
ACT	acetylthiocholine iodide
ACTH	adenocorticotropic hormone
ADI	acceptable daily intake
ai	active ingredient
ALD	aldrin epoxidase
ALT	alanine aminotransferase
AR	androgen receptor
ARfD	acute reference dose
AST	aspartate aminotransferase
AUC	area under the curve of concentration–time
$BMDL_{10}$	benchmark-dose lower 90% confidence level
BROM	bromocriptine
bw	body weight
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
CCN	Codex classification number (for compounds or commodities)
CCPR	Codex Committee on Pesticide Residues
CI	confidence interval
CSAF	chemical-specific assessment factor
C_{max}	maximum concentration
DβH	dopamine-β-hydroxylase
DA	dopamine
DACT	diaminochlorotriazine
DEA	deethyl-atrazine
DHT	dihydroxytestosterone
DIA	deisopropyl-atrazine
DMCPA	1,2-dimethylcyclopropane-dicarboxylic acid
DMSO	dimethyl sulfoxide
DNCB	1-chloro-2,4-dinitrobenzene
DNTB	dithiobisnitrobenzoate
E2	estradiol
EC_{50}	the concentration of agonist that elicits a response that is 50% of the possible maximum
ECG	electrocardiogram
ECOD	7-ethoxycoumarin-deethylase
EH	epoxide hydrolase

ER	estrogen receptor
EROD	7-ethoxyresorufin-deethylase
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
FACS	fluorescence-activated cell sorting
FAO	Food and Agricultural Organization of the United Nations
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FOB	functional observational battery
GAP	good agricultural practice
GC	gas chromatography
GGT	gamma-glutamyltransferase
GEMS/Food	Global Environment Monitoring System–Food Contamination Monitoring and Assessment Programme
GnRH	gonadotropin-releasing hormone
GSD	geometric standard deviation
GST	glutathione *S*-transferase
HA	hypothalamic amenorrhoea
hCG	human chorionic gonadotrophin hormone
HPLC	high-performance liquid chromatography
HPLC-UV	high-performance liquid chromatography with ultraviolet light detector
HR	highest residue in the edible portion of a commodity found in trials used to estimate a maximum residue level in the commodity
HR-P	highest residue in a processed commodity calculated by multiplying the HR of the raw commodity by the corresponding processing factor
IC_{50}	concentration required to inhibit activity by 50%
IEDI	international estimated daily intake
IESTI	international estimate of short-term dietary intake
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint Meeting on Pesticide Residues
JMPS	Joint FAO/WHO Meeting on Pesticide Specifications
LC	liquid chromatography
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LE	Long-Evans
LH	luteinizing hormone
LOAEC	lowest-observed-adverse-effect concentration
LOAEL	lowest-observed-adverse-effect level

LOD	limit of detection
LOQ	limit of quantification
MAFF	Ministry of Agriculture, Fisheries and Food
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MEQ	methylethoxyquin
MIC	minimum inhibitory concentration
MMAD	mass median aerodynamic diameter
MRL	maximum residue limit
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MTD	maximum tolerated dose
NCE	normochromatic erythrocytes
NMR	nuclear magnetic resonance
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OECD	Organization for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
OR	odds ratio
PCE	polychromatic erythrocytes
PCPS	polycystic ovarian syndrome
PHI	pre-harvest interval
PPARα	peroxisome proliferator-induced receptor alpha
ppm	parts per million
PROD	7-pentoxyresorufin *O*-depentylase
QA	quality assurance
STMR	supervised trials median residue
STMR-P	supervised trials median residue in a processed commodity calculated by multiplying the STMR of the raw commodity by the corresponding processing factor
T3	triiodothyronine
T4	thyroxin
TBPS	*tert*-butylbicyclophosphorothionate
TH	tyrosine hydroxylase
TIPA	triisopropylammonium
TLC	thin-layer chromatography
THPI	1,2,3,6-tetrahydrophthalimide
TRR	total radiolabelled residue
TSH	thyroid-stimulating hormone

TMDI	theoretical maximum daily intake
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 9-18 September 2008.

Six of the substances evaluated by the WHO Core Assessment Group (azoxystrobin, chlorantraniliprole, mandipropamid, prothioconazole, spinetoram and spirotetramat) were evaluated for the first time. Six compounds (buprofezin, hexythiazoz, flusilazole, procymidone, profenofos) were re-evaluated within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR). Reports and other documents resulting from previous Joint Meetings on Pesticide Residues are listed in Annex 1.

The report of the Joint Meeting has been published by the FAO as *FAO Plant Production and Protection Paper 193*. 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 2008, 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 2008 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

AZOXYSTROBIN

First draft prepared by
P.V. Shah[1] and David Ray[2]

[1]United States Environmental Protection Agency, Office of Pesticide Programs, Washington DC, USA; and
[2]School of Biomedical Sciences, University of Nottingham, Queens Medical Centre, Nottingham, England

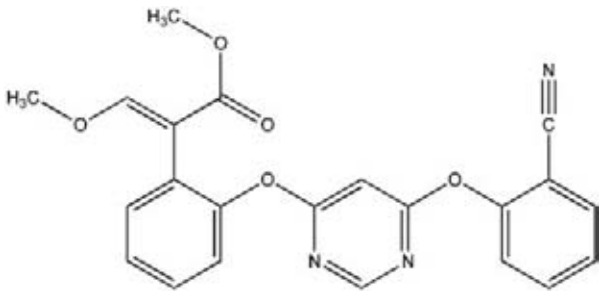
Explanation

Azoxystrobin is the ISO approved name for methyl (*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate, (IUPAC), for which the CAS No. is 131860-33-8. Azoxystrobin is a β-methacrylate compound that is structurally related to the naturally occurring strobilurins, which are compounds derived from some fungal species. Azoxystrobin is a broad-spectrum, systemic fungicide that acts by inhibiting electron transport in pathogenic fungi. It has the ability to provide

protection against the fungal diseases caused by *Ascomycota*, *Deuteromycota*, *Basidiomycota* and *Oomycota* groups.

Azoxystrobin has not been evaluated previously by JMPR and was evaluated by the present Meeting at the request of the Fortieth Session of the Codex Committee on Pesticide Residues (CCPR). All pivotal studies with azoxystrobin were certified as complying with good laboratory practice (GLP).

Figure 1. Chemical structure of azoxystrobin



Evaluation for acceptable daily intake

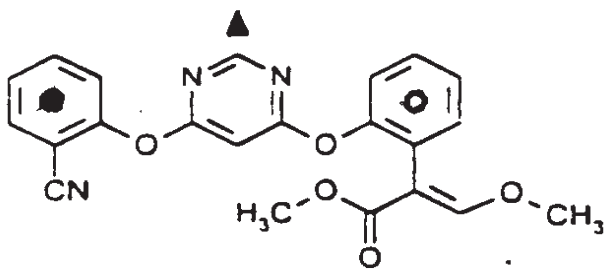
Unless otherwise stated, studies evaluated in this monograph were performed by GLP-certified laboratories and complied with the relevant Organization for Economic Co-operation and Development (OECD) and/or United States Environmental Protection Agency (EPA) test guideline(s).

1.1 Biochemical aspects: absorption, distribution and excretion

Rats

The absorption, distribution, and elimination of azoxystrobin was studied after oral dosing of azoxystrobin radiolabelled with ^{14}C as shown in Figure 2.

Figure 2. Position of the radiolabel on azoxystrobin used in pharmacokinetic studies in rats



▲ Denotes position of pyrimidinyl ring label

❍ Denotes position of phenylacrylate ring label

● Denotes position of cyanophenyl ring label

Studies using whole-body autoradiography were conducted using azoxystrobin labelled with ^{14}C in either the cyanophenyl, pyrimidinyl or phenylacrylate ring (see Figure 2). The excretion and

tissue distribution of radioactivity was investigated for 48 h in male and female rats given a single dose of azoxystrobin at 1 mg/kg bw by gavage. Treated rats were housed in metabolism cages to facilitate the collection of urine, faeces, exhaled air and volatiles. One male and one female rat receiving azoxystrobin radiolabelled in each position were killed at 24 h and 48 h after dosing. Each carcass was frozen and sectioned in preparation for whole-body radiography.

About 89% and 86% of the administered dose of [^{14}C]pyrimidinyl-labelled azoxystrobin was excreted within 48 h in the urine and faeces of male and female rats, respectively. Most of the radioactivity was excreted in the faeces, with < 17% in the urine. The male and female rats treated with [^{14}C] phenylacrylate-labelled azoxystrobin excreted about 80% and 97% of the administered dose within 48 h, respectively. Most of the radioactivity was excreted via the faeces with < 21% in the urine. At 48 h, males and females, excreted approximately 0.01% of the administered dose as carbon dioxide trap and approximately 0.01% as volatile metabolites. The male and female rats treated with]^{14}C]cyanophenyl-labelled azoxystrobin excreted about 95% and 98% of the administered dose within 48 h, respectively. Most of the radioactivity was excreted via the faeces, with < 16% in the urine. At 48 h, males and females excreted small amounts of radioactivity as carbon dioxide (< 0.3%) and as volatile metabolites (0.01%). For all radiolabels, the distribution of radioactivity was similar in males and females, as shown by whole-body autoradiography. At 24 h, most of the radiolabel was present in the alimentary canal, moderate amounts in the kidneys and small amounts in the liver. Forty-eight hours after dosing, the whole-body autoradiography results showed a marked reduction in radioactivity.

The results of these studies indicated that there were no significant differences between the rates and routes of excretion or tissue distribution of azoxystrobin labelled in one of three positions. No sex-related difference in excretion profile was evident. Minor differences in excretion were primarily due to the small numbers of rats used in the study. No significant differences in the amount of radioactivity recovered in the exhaled air and as volatiles were observed between the three radiolabels or between sexes. On the basis of the results of this study, other studies of excretion and tissue retention were conducted using only pyrimidinyl-labelled azoxystrobin (Lythgoe & Howard, 1993a).

In toxicokinetic studies, groups of male and female Alpk:APfSD rats (five to eight per group, depending on experiment) were given azoxystrobin (purity, 99%) with or without pyrimidinyl label as a single dose at 1 or 100 mg/kg bw by gavage or as 14 repeated doses of 1 mg/kg bw per day. Biliary metabolites were assessed using rats with cannulated bile ducts given a single dose at 100 mg/kg bw by gavage. The vehicle was polyethylene glycol (PEG 600) at 4 ml/kg bw. Treated rats were housed in stainless steel metabolism cages for 7 days. Urine was collected at 6 h, and urine and faeces were collected separately at 12, 24, 36, 48 h and at 24 h intervals until 7 days after dosing. At each collection, cages were rinsed with water and cage-washing collected together with the urine. At the end of the study, cages were thoroughly rinsed with ethanol/water (1 : 1 v/v) and retained for radiochemical analysis. Carbon dioxide and volatiles were trapped. After 7 days, various organs and tissues were removed and analysed for radioactivity. Recovery of radioactivity in selected tissues and excreta of rats treated with azoxystrobin at a single lower or higher dose and after repeated doses of azoxystrobin for 7 days is shown in Table 1.

For rats receiving a single lower dose (1 mg/kg bw), total excretion of radioactivity (urine, faeces, and cage wash) was 93.75% and 91.44% for males and females, respectively over the 7 days. Most (> 85%) of the urinary and faecal excretion took place during the first 36 h after dosing. In these rats, about 83.2% and 72.6% of the administered dose was excreted in the faeces of males and females within 7 days, respectively, and about 10.2% and 17.9% of the administered dose was excreted in the urine of the males and females within 7 days, respectively. Approximately 0.34% and 0.31% of the administered dose was found in the carcass and tissues within 7 days after dosing in males and females, respectively. For rats at this dose (1 mg/kg bw), the highest concentrations of radiolabel were found in the liver (mean for males and females, 0.009 µg equivalents/g) and in

the kidneys (males, 0.027 µg equivalents/g; and females, 0.023 µg equivalents/g). At termination, the total concentration of radioactivity in blood was 0.004 µg equivalents/g for males and females. Less than 0.6% of the administered dose was recovered in the expired air (Jones, 2004; Lythgoe & McAsey, 1995, 1993).

For rats receiving the single higher dose (100 mg/kg bw), total excretion of radioactivity (urine, faeces, and cage wash) was 98.29% and 97.22% for males and females, respectively, over the 7 days. Most (> 82%) of the urinary and faecal excretion took place during the first 48 h after dosing. At this dose, about 89.37% and 84.53% of the administered dose was excreted in the faeces of the males and females within 7 days, respectively, and about 8.54% and 11.54% of the administered dose was excreted in the urine of the males and females within 7 days, respectively. Approximately 0.33% and 0.33% of the administered dose was found in the carcass and tissues within 7 days after dosing in males and females rats, respectively. At this higher dose, the highest concentrations of radiolabel were found in the kidneys (males, 1.373 µg equivalents/g; and females, 1.118 µg equivalents/g) and in the liver (males, 0.812 µg equivalents/g; and females, 0.714 µg equivalents/g). At termination, the total concentration of radioactivity in blood was 0.389 µg equivalents/g for males and 0.379 µg equivalents/g for females (Lythgoe & Howard, 1995, 1993b).

Eight male and female rats were given 14 consecutive daily oral doses of unlabelled azoxystrobin at 1 mg/kg bw followed by a single oral dose of [^{14}C]pyrimidinyl-labelled azoxystrobin at 1 mg/kg bw. For the repeated doses, about 89.1% and 86.5% of the administered dose was excreted in the faeces of the males and females rats within 7 days, respectively, and about 12.5% and 17.0% of the administered dose was excreted in the urine of the males and females rats within 7 days, respectively. In males and females, excretion of radioactivity was rapid, with > 96% being excreted during the first 48 h. Approximately 0.62% and 0.39% of the administered dose was found in the carcass and tissues within 7 days after dosing in male and female rats, respectively. For the repeated dose, the highest concentrations of azoxystrobin-derived radioactivity were found in the kidneys (males and females, < 0.04 µg equivalents/g). The concentrations found in the liver were 0.02 and 0.01 µg equivalents/g for males and females, respectively. At termination, the total concentration of radioactivity in blood was 0.01 µg equivalents/g for males and females (Lythgoe & Howard, 1993c).

Bile-duct cannulated rats were given azoxystrobin radiolabelled in either the pyrimidinyl, cyanophenyl or phenylacrylate rings at 100 mg/kg bw by gavage. Comparison of the rates and routes

Table 1. Recovery of radioactivity in tissues and excreta of rats given ^{14}C-labelled azoxystrobin orally

Sample analysed	Recovery (% of administered dose)					
	Single lower dose		Repeated lower dose		Single higher dose	
	Male	Female	Male	Female	Male	Female
Expired air	NP	NP	NP	NP	NP	NP
Tissues	0.11 ± < 0.01	0.08 ± < 0.01	Approx. 0.12	Approx. 0.08	Approx. 0.07	Approx. 0.05
Carcass	0.23 ± 0.02	0.23 ± 0.06	0.50 ± 0.08	0.31 ± 0.05	< 0.26	< 0.27
Body, total	0.34	0.31	0.62	0.39	0.33	0.33
Cage wash	0.33 ± 0.13	0.93 ± 0.58	0.50 ± 0.5	0.10 ± 0.1	0.38 ± 0.13	1.15 ± 0.78
Urine	10.19 ± 1.53	17.89 ± 3.50	12.50 ± 3.4	17.00 ± 2.7	8.54 ± 1.03	11.54 ± 1.42
Faeces	83.24 ± 1.52	72.62 ± 5.40	89.10 ± 5.9	86.5 ± 1.7	89.37 ± 3.99	84.53 ± 1.98
Excreta; total	93.75	91.44	102.1	103.6	98.29	97.22
Total recovery	94.1	91.75	102.72	103.99	98.61	97.54

From Lythgoe & Howard (1993a, 1993b) and Lythgoe & McAsey (1993)
NP, not performed in the studies of excretion/distribution.

of excretion and the profile of the metabolites showed (as previously) that there were no significant differences in the metabolism of the three differently labelled forms, thus indicating that there was minimal cleavage of the ether linkages between the aromatic rings. Experiments designed to identify metabolites were therefore conducted in bile-duct cannulated rats given [^{14}C]pyrimidinyl labelled azoxystrobin by gavage. In the bile-duct cannulated rats, excreta, bile, and cage wash were collected at 6, 12, 24, 36, and 48 h and stored at –20 °C. Samples of bile, faeces and urine were collected between 0 h and 48 h and pooled. Samples for males and females were separated. Urine and faeces were collected at up to 168 h after dosing from rats given the single dose (higher or lower) and from rats receiving repeated doses for 14 days, and were used for quantification of metabolites. Some bile samples were enzymatically digested using cholylglycine hydrolase at 30 units/ml, pH 5.6 at 37 °C overnight. Metabolites were identified using various analytical techniques, such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), proton nuclear magnetic resonance spectroscopy (NMR) and mass spectrophotometry (MS).

On the basis of biliary excretion data for rats given a single dose of either [^{14}C]pyrimidinyl-, [^{14}C]phenylacrylate-, or [^{14}C]cyanophenyl-labelled azoxystrobin at 100 mg/kg bw, 74.4% (males) and 80.7% (females) of the pyrimidinyl-derived radioactivity was excreted in the bile after 48 h. For the cyanophenyl-derived radioactivity, 56.6% and 62.5% was excreted in the bile of males and females, respectively. For the phenylacrylate-derived radioactivity, 64.4% (males) and 63.6% (females) was excreted in the bile. Quantitatively, there were no significant differences in biliary excretion between males and females.

Azoxystrobin was found to undergo extensive metabolism in rats. A total of 15 metabolites were detected in the excreta and subsequently identified. Seven additional metabolites were detected but not identified. None of the unidentified metabolites represented more than 4.9% of the administered dose. The quantitative data for the various metabolites in the faeces, urine and bile of rats receiving a single dose of azoxystrobin at 100 mg/kg bw are shown in Table 2. The mass balance for the study of metabolite identification indicated that a substantial percentage of the administered radiolabel (45.6–73.6%) was unaccounted for, although the studies of excretion showed total recovery of 91.75–103.99%, with 72.6–89.3% being in the faeces. The percentage of unaccounted-for radiolabel was especially notable in the groups receiving a single lower dose and a repeated lower dose. The study authors indicated that the variable efficiency in recovery could be explained by the fact that, for metabolite identification, faeces were extracted with acetonitrile which allowed partitioning of the parent compound when it was present in the faeces (i.e. rats receiving the higher dose). For the groups receiving a single lower dose or repeated lower dose (where quantities of the parent compound were minimal), most of the faecal radiolabel was associated with polar metabolites that would not be present in the acetonitrile extract. The resulting concentration of radiolabel in the extract would, therefore, be very low. For the group receiving the higher dose, greater amounts of parent compound were left unabsorbed, thereby resulting in greater amounts of parent compound available for partitioning into the acetonitrile extract.

The glucuronide conjugate (metabolite V) was the most prevalent biliary metabolite in both males (29.3%) and females (27.4%). Metabolite I (parent compound) was not detected in the bile. Each of the other biliary metabolites accounted for between 0.9% and 9.0% of the administered dose. In the bile-duct cannulated rats, about 15.1% and 13.6% of the faecal radioactivity was metabolite I (parent compound) in male and female rats, respectively. No parent compound was detected in the urine of bile-duct cannulated male and female rats. The predominant metabolite in the urine of the bile-duct cannulated rats was unidentified metabolite 2, which accounted for about 1.8% and 2.0% of the administered dose in male and female rats, respectively.

There was no evidence for a dose-influencing metabolism, but a sex-specific difference in biotransformation was observed, with females producing more metabolites than did males. Biotransformation was unaffected by dose. The study authors suggested that absorption was dose-dependent.

The oral absorption at 1 mg/kg bw was nearly complete (100%) since no parent compound was detected. The oral absorption at the higher dose (100 mg/kg bw) was estimated to be approximately 74–81% since about 19–26% of the parent compound was detected. However, it is difficult to estimate the true oral absorption value owing to poor recoveries after extraction, especially at the lower dose.

The proposed metabolic pathway for azoxystrobin in rats is shown in Figure1. There were two principal metabolic pathway: hydrolysis to the methoxyacid, followed by glucuronide conjugation to give metabolite V; and glutathione conjugation of the cyanophenyl ring followed by further metabolism via a number of intermediates (VI, VII, and VIII) to the mercapturic acid metabolite IX. Azoxystrobin was also hydroxylated at the 8 and 10 positions on the cyanophenyl ring followed by glucuronide conjugation (metabolites II, III, IVa and IVb). There were several minor pathways involving the acrylate moiety, resulting in formation of the metabolite XIII and XIV. Three metabolites (X, XII, and XV) arising via the cleavage of the ether linkages were identified (Lappin & Gledhill, 1994).

In an additional metabolism study, [^{14}C]cyanophenyl-labelled azoxystrobin was given to bile-duct cannulated and non-cannulated rats at a dose of 100 mg/kg bw. Samples of urine, faeces and bile were collected for up to 72 h. The purpose of this study was to reevaluate certain plant and goat metabolites that were previously not identified in rats and further elucidate the metabolic pathway of azoxystrobin in rats.

Table 2. Identification and distribution of metabolites in bile-duct cannulated rats given azoxystrobin as a single dose at 100 mg/kg bw by gavage

Metabolite	Recovery (% of administered dose)							
	Males				Females			
	Faeces	Bile	Urine	Total	Faeces	Bile	Urine	Total
I (azoxystrobin)	15.1	—	—	15.1	13.6	—	—	13.6
II	—	6.5	—	6.5	0.1	6.8	0.3	7.2
III	—	—	0.1	0.1	—	1.7	—	1.7
IVA+IVA [a]	—	6.8	—	6.8	—	9.0	0.3	9.3
IVb + IVb [a]	—	—	—	—	0.1	1.4	0.2	1.7
V	—	29.3	0.1	29.4	—	27.4	1.7	29.1
VII + XI [a]	—	7.0	—	7.0	—	1.6	0.3	1.9
VIII = XIV [a]	—	3.2	0.1	3.3	—	6.1	0.3	6.4
IX	—	4.5	—	4.5	0.1	2.4	0.4	2.9
X	—	—	—	—	—	4.8	0.4	5.2
XIII	—	2.8	Trace	2.8	—	0.9	Trace	0.9
XV	0.2	4.1	0.3	4.6	0.2	1.5	0.4	2.1
Unidentified No. 1	—	4.4	—	4.4	—	2.1	—	2.1
Unidentified No. 2	—	2.5	1.0	3.5	—	1.3	1.8	3.1
Unidentified No. 3	—	1.1	0.2	1.3	—	1.1	0.4	1.5
Unidentified No. 4	—	—	—	—	—	—	0.1	0.1
Unidentified No. 5	—	—	0.1	0.1	—	1.3	0.3	1.6
Unidentified No. 6	0.1	—	0.1	0.2	0.1	4.4	—	4.5
Total identified	15.4	72.2	2.0	89.6	14.2	73.8	6.9	95.0

From Lappin & Gledhill (1994)

[a] Metabolites could not be fully resolved by high-performance liquid chromatography (HPLC) and individual quantitation was not possible.

Three further metabolites, previously detected in either plants or goats, were identified.

The IUPAC names of the selected metabolites are given in Table 3. Compound 13, resulting from cleavage of the diphenyl ether link, was detected in the bile and urine as the glucoronide conjugate at a concentration of up to 1.8% of the administered dose. Compound 20 was also detected in the bile and urine at a concentration of up to 1.3%. Compound 35 was detected in the urine, faeces and bile at a concentration of up to 0.6%. Compounds 24 and 30 were not detected (Joseph et al., 1995).

2. Toxicological studies

2.1 Acute toxicity

The acute toxicity of azoxystrobin is summarized in Table 4.

Table 3. IUPAC names of the selected metabolites of azoxystrobin reevaluated in a study in rats

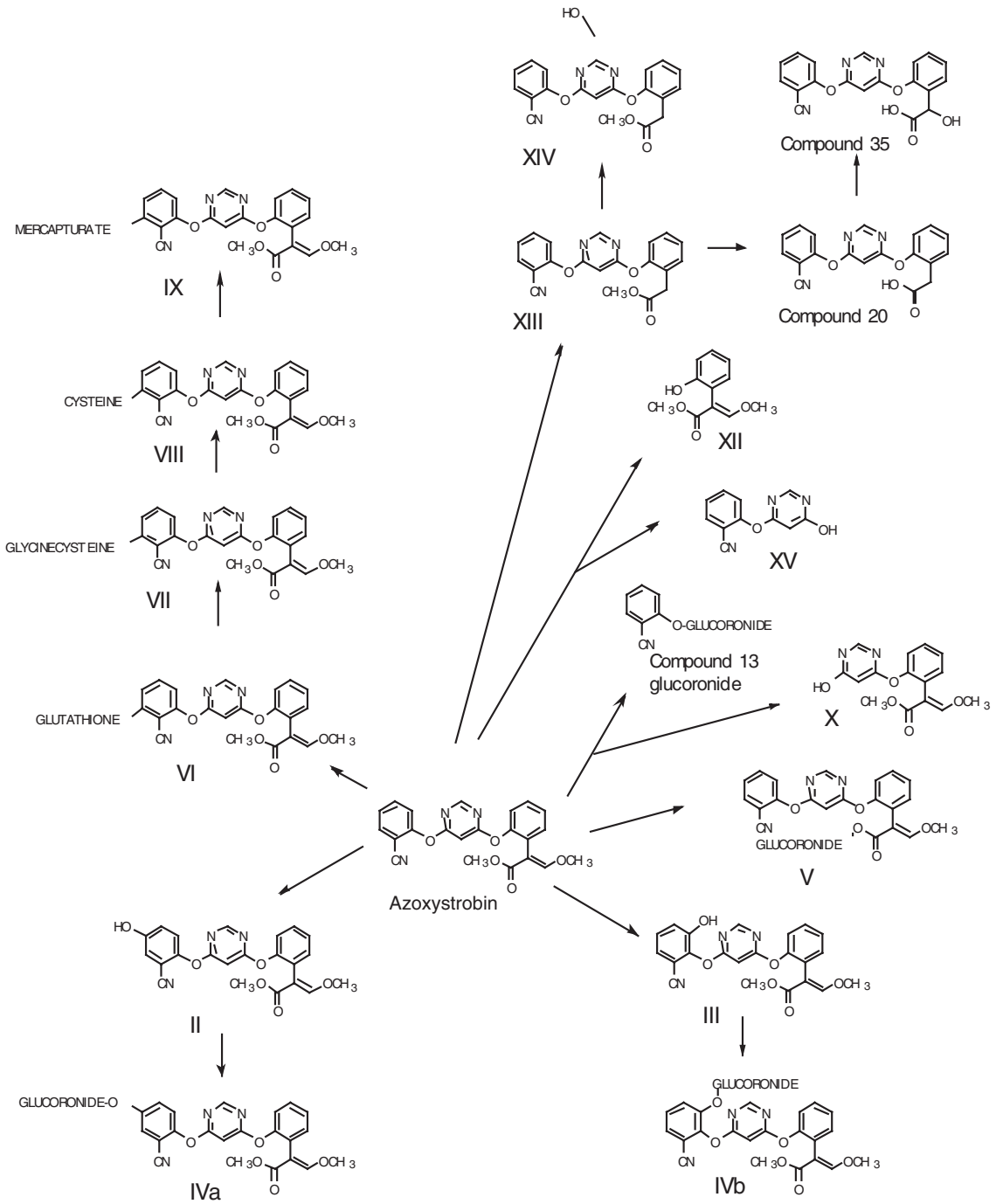
Compound No.	Code No.	IUPAC name
13	R71395	2-hydroxybenzonitrile
20	R400050	{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}acetic acid
24	R400753	Methyl 2-{2[6-(2-cyanophenoxy)pyrimidin-4-yloxy] phenyl}-glycolate
30	R402173	2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] benzoic acid
35	R402987	2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}glycolic acid

From Joseph et al. (1995)
IUPAC, International Union of Pure and Applied Chemistry

Table 4. Acute toxicity of azoxystrobin

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Reference
Mice	CD-1	Males and females	Oral	> 5000	—	Robinson (1991b, 1995b)
Rats	Wistar (Crl: (WI) BR)	Males and females	Oral	> 5000	—	Robinson, (1991a, 1995a)
Rats	Wistar (Crl: (WI) BR)	Males and females	Dermal	> 2000	—	Robinson (1991c, 1995c)
Rat	Wistar Alpk: APfSD	Males and females	Inhalation (4 h, nose-only)	—	Females: 0.698 Males: 0.962	Parr-Dobrzanski (1992, 1995)
Rat	Wistar Alpk:APfSD		Inhalation	—	≥ 4.7	Pinto (1997)
Rabbits	New Zealand White	Females	Dermal irritation	Slight irritation	—	Robinson (1991d, 1995d)
Rabbit	New Zealand White		Ocular irritation	Slight irritation		Robinson (1991f, 1995e)
Guinea-pig	Dunkin-Hartley		Dermal sensitization	Not sensitizing		Robinson (1991g, 1995f)

Figure 3. Proposed metabolic pathway of azoxystrobin in rats



Note: Metabolite numbers are shown as roman numerals.

(a) Oral toxicity

Mice

Groups of five male and five female young adult CD-1 mice were given azoxystrobin (purity, 95.2%) as a single dose at 0, or 5000 mg/kg bw by gavage in corn oil. Treated mice were subjected to gross necropsy after 14 days. Two male mice died owing to dosing accidents and were replaced. There were no treatment-related mortalities. Clinical observations were confined to slight piloerection and slight urinary incontinence in some mice. All clinical signs had regressed by day 6. There were no significant treatment-related clinical signs, necropsy findings or changes in body weight. The oral median lethal dose (LD_{50}) for azoxystrobin in mice was > 5000 mg/kg bw for males and females (Robinson, 1991b, 1995b).

Rats

Groups of five male and five female young adult Wistar rats (Crl:(WI)BR) rats were given azoxystrobin (purity, 95.2%) as a single dose at 0 and 5000 mg/kg bw by gavage in corn oil. Treated rats were subjected to gross necropsy after 14 days. One female rat died on day 2 from a dosing accident and was replaced. The female that died on study day 2 had excess watery fluid in the thoracic cavity consistent with inappropriate administration of the test material. All rats lost weight initially, due to pre-dose fasting, but most had exceeded their initial weight by day 8, and continued to gain weight until the end of the study. There were no significant treatment-related clinical signs, necropsy findings or changes in body weight. There were no treatment-related deaths. The oral LD_{50} of azoxystrobin in rats was > 5000 mg/kg bw for males and females (Robinson, 1991a, 1995a).

(b) Dermal toxicity

Rats

Five male and five female young adult Wistar (Crl:(WI)BR) rats were exposed dermally to azoxystrobin (purity, 95.2%) at 2000 mg/kg bw as a paste in corn oil applied to approximately 10% of the (shaved) body surface area. The test substance was maintained in contact with the skin for 24 h using an occlusive dressing. The rats were observed for 14 days. Body weights were recorded at intervals throughout the study. All rats were subjected to a post-mortem examination at termination. Slight skin irritation (slight erythema) was observed during the study, but there were no significant signs of systemic toxicity and none of the rats died. All rats lost weight initially, but all had exceeded their initial weights by day 6. Post-mortem examination did not reveal any treatment-related pathological effects. The dermal LD_{50} of azoxystrobin in rats was > 2000 mg/kg bw for males and females (Robinson, 1991c, 1995c).

(c) Exposure by inhalation

Rats

In a study of acute toxicity after inhalation, groups of five male and five female young adult Wistar rats (Alpk:APfSD) were exposed nose-only to azoxystrobin (purity, 96.2%) for 4 h at a concentration of 0.2, 0.5, or 0.8 mg/l. One additional group of five male rats was exposed to azoxystrobin at 1.0 mg/l. the rats were then observed for 14 days. The mean measured particulate concentrations were 0, 0.257, 0.511, 0.767 or 1.010 mg/l, which were chemically analysed as 0, 0.242, 0.481, 0.717 or 0.968 mg/l. Atmospheres generated had mean aerodynamic particle sizes of 1.13, 1.17, 1.35 and 1.17 µm. No mortality was observed at 0.2 mg/l. Mortality occurred at 0.5 mg/l (one male and one female), 0.8 mg/l (one male and three females) and 1.0 mg/l (three males). Most rats exposed to azoxystrobin at 0.5, 0.8, or 1.0 mg/l developed slow deep breathing, auditory hypoesthaesia, and breathing irregularities during and up to 4 days after exposure. In addition, many rats had a splayed

gait and reduced splay reflex immediately after exposure. Surviving rats showed rapid recovery, and all treatment-related clinical signs had disappeared by day 7. Body weight was reduced in surviving rats in all groups after exposure, but by day 8 most rats were gaining weight and had exceeded their initial weights. All rats that died during exposure had dark red or mottled lungs. No other treatment-related effects were observed.

The median lethal concentration (LC_{50}) of azoxystrobin at 4 h in rats was calculated to be 0.698 mg/l for females and 962 mg/l for males (Parr-Dobrzanski, 1992, 1995).

In a second study of acute toxicity after inhalation, groups of five male and five female young adult Wistar rats (Alpk: APfSD) were exposed nose-only to azoxystrobin technical grade active ingredient (purity, 96.2%) for 4 h at a target concentration of > 3.7 mg/l. The rats were then observed for 14 days. The mean measured particulate concentration was 4.7 mg/l. Atmospheres generated had mean aerodynamic particle size of 14.7 µm. The range of particles size was too large for a study of toxicity after inhalation. No mortality was observed during the study. After exposure, some rats had wet fur, piloerection and hunched posture, which subsided by day 4. Body weights of four male and four female rats were increased by day 8 and these rats continued to gain weight until the end of the study. No other treatment-related effects were observed at gross necropsy.

The LC_{50} of azoxystrobin technical grade active ingredient at 4 h in rats was > 4.7 mg/l for males and females (Pinto, 1997).

(d) Dermal irritation

In a study of primary dermal irritation, six young adult female New Zealand White rabbits were dermally exposed to 0.5 g of azoxystrobin (purity, 95.2%), moistened with distilled water, for 4 h. The treated area was covered by an occlusive dressing. The application site was washed after removal of the dressing and dermal irritation was assessed after 30–60 min and then daily for up to seven days. Very slight erythema and oedema were present for three days after dosing in one rabbit, and for 1 h in another. No other signs of irritation were observed. The Meeting concluded that azoxystrobin was a slight dermal irritant in rabbits given a single application for 4 h. The mean erythema and mean oedema scores over the first 3 days were calculated to be 0.2 and 0.2, respectively (Robinson, 1991d, 1995d).

(e) Ocular irritation

In a study of primary eye irritation, 0.1 ml of azoxystrobin (purity, 95.2%), was instilled into the conjunctival sac of one eye of each of six young adult female New Zealand White rabbits. The initial pain reaction was assessed immediately after treatment. Irritation was scored by the method of Draize at 1–2 h, and 1, 2, and 3 days after exposure. The test material induced slight to moderate erythema and slight chemosis in all rabbits within 1 h, but the effects resolved within 48 h after treatment. Additional signs of irritation included slight mucoid and Harderian discharge and partial haemorrhaging of the nictitating membrane. These effects had completely regressed 2 days after dosing. The Meeting considered that azoxystrobin was slightly irritating (class 3 on a 1–8 scale) to the eyes of rabbits (Robinson, 1991e, 1995e).

(f) Sensitization

In a study of dermal sensitization with azoxystrobin (purity, 95.2%) mixed with corn oil, young male and female Dunkin-Hartley guinea pigs were tested using the maximization method of Magnusson & Kligman. For the main study, 10 female guinea-pigs were assigned to a control group, and 10 female guinea-pigs to the treatment group. In this study, the test concentrations chosen were 10% for intradermal induction, 64% for topical induction, and 37% or 67% for the challenge. Skin reactions at the challenge sites were observed at 24 h and 48 h after removal of the patch. No mortalities or

clinical signs of toxicity were observed in the study. Challenge of previously induced guinea-pigs with a 67% or a 30% w/v preparation of azoxystrobin in corn oil caused light brown staining at some challenge sites, but this did not obscure the assessment of any erythematous response that may have been present. There were no signs of dermal reactions in any guinea-pigs in the induction or challenge phase. In a study designed to provide a positive control, challenge of previously induced guinea-pigs with a 10% w/v dilution of a 40% w/v aqueous formaldehyde solution elicited an extreme skin sensitization response. The Meeting concluded that azoxystrobin was not a dermal sensitizer in guinea-pigs, as determined by the maximization method (Robinson, 1991g, 1995f).

2.2 Short-term studies of toxicity

Rats

In a 21-day study of dermal toxicity after repeated doses, groups of five male and five female Wistar (Alpk:APfSD) rats received dermal applications of azoxystrobin (purity, 96.2%) at a dose of 0, 200, 500 or 1000 mg/kg bw per day formulated in deionized water, for 6 h/day, for a total of 21 days over a 30-day period. The hair was clipped from the back of each rat before the first application, then periodically as required. The application site was then wrapped in occlusive gauze bandage covered by a patch of plastic film and secured with polyvinylchloride (PVC) tape for 6 h. After the exposure, the gauze and tape were removed and the application site was cleansed free of any residual test material, using a clean swab of cotton wool soaked in warm water, and dried. The control group received distilled water applied with the same method. The rats were observed twice per day for signs of mortality, morbidity, toxicity, and the presence of dermal irritation. Dermal reactions at the application site were scored daily (before dosing) using the Draize score. Body weight was measured before dosing then daily thereafter. Food consumption was calculated on a daily basis. Blood was taken at the end of the study, and the standard test parameters were examined. At the end of the study, all rats were examined grossly post mortem. Testes, kidneys and liver were weighed. The adrenals, brain, kidneys, liver, testes, epididymides, treated skin, and untreated skin were removed and examined microscopically. This study was conducted in accordance with GLP regulations.

No mortality was observed and there were no significant treatment-related clinical abnormalities. There were no treatment-related effects on body weight, food consumption, organ weights, clinical biochemistry, or haematology. There were no treatment-related pathological abnormalities. Abdominal scabs and scabs at the edge of the application area were observed in all groups of females and were attributed to the bandaging method and were not of toxicological significance.

The no-observed-adverse-effect level (NOAEL) for systemic toxicity and dermal irritation with azoxystrobin was 1000 mg/kg bw per day (the highest dose tested). A lowest-observed-adverse-effect level (LOAEL) was not identified (Robinson, 1994).

In a 90-day study of toxicity, groups of 12 male and 12 female Wistar-derived (Alpk:APfSD) rats were given diets containing azoxystrobin (purity, 95.2%) diet at a concentration of 0, 200, 2000 or 4000 ppm (equal to 0, 20.4, 211.0 or 443.8 mg/kg bw per day for males and 0, 22.4, 223.0 or 448.6 mg/kg bw per day for females) for 13 weeks. The groups given diets at 4000 ppm were initially given diets at 6000 ppm, but this concentration was reduced after 15 days owing to reduced food consumption and a marked reduction in growth. After 5 days on control diet, this group was subsequently fed diets containing azoxystrobin at 4000 ppm for the rest of the study. Diets were prepared at the initiation of the study and stored frozen. Stability, homogeneity and dietary concentrations were confirmed analytically. Rats were inspected daily for signs of toxicity and mortality, with detailed cage-side observations done weekly. Body weight and food consumption were measured weekly. At termination, blood was taken for haematological and clinical chemistry analysis. Urine analysis and

ophthalmoscopic examinations were performed during the week of termination. All rats that died and those that were sacrificed on schedule were given gross pathological examinations and selected organs were weighed. Selected tissues were collected for histological examination.

Diets were stable for 64 days at room temperature. The test article homogeneity results were within the acceptable range (–0.8 to +1.4% deviation from the mean). The analysis of test substance concentration indicated that the measured concentrations of azoxystrobin ranged from 92% to 111% of the target concentrations.

No mortality occurred during the study. Distended abdomen was seen in males and females at 2000 and 4000 ppm, consistent with local gastrointestinal disturbances and reduced nutritional status. At termination, males (11 out of 12) and females (10 out of 12) in the group at 4000 ppm appeared to be of small size compared with rats in the control group or at 200 ppm. No other treatment-related clinical signs of toxicity were observed. Final body weights of males and females at 4000 ppm were reduced by 32% and 18%, respectively, and final body weights of males and females at 2000 ppm were reduced by 18% and 11%, respectively. Food consumption and food efficiency were reduced in males and females at 4000 ppm, particularly during weeks 1–2 or weeks 1–4. However, by the end of the study, food efficiency of females at 4000 ppm was not significantly reduced compared with that of controls.

The results of ophthalmological examination of rats at 4000 ppm were comparable to those for rats in the control group. Minimal reductions in haemoglobin, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) were observed only in females at 4000 ppm. Minor but statistically significant reductions in MCV at 200 and 2000 ppm and MCH at 2000 ppm were observed in females. Leukocyte count was statistically significantly increased in females at 4000 ppm. Platelet counts were slightly decreased in males and females at 4000 ppm. Clotting parameters were not affected. The changes in haematological parameters were small; these changes were therefore not considered to be toxicologically significant. Changes in clinical chemistry parameters such as reduced cholesterol (males), glucose (females), decreased triglycerides (males and females), and decreases in some plasma enzyme activities (males and females) were observed at 4000 ppm. All these findings were less marked in the groups at 2000 ppm and were absent in the groups at 200 ppm. The total urinary protein of males at 4000 ppm was reduced. Blood was present in the urine of males in the control group and in a number of male and female rats at 2000 and 4000 ppm. Increases in liver and kidney weights adjusted for body weight in rats at 2000 and 4000 ppm were attributable to treatment with azoxystrobin. Changes in organ weights were accompanied by histopathological findings in two males at 4000 ppm. Treatment-related effects in these males included marked elevations in total bilirubin, cholesterol, triglycerides, and plasma enzyme activities. The effect on the liver of these two rats was observed microscopically as proliferation of the intrahepatic bile duct/ductiles and oval cells. Hepatocellular hyperplasia and an enlarged hepatic lymph node were observed in one of the two males. There was a reduction in renal tubular basophilia in males at 4000 ppm.

The LOAEL was 2000 ppm, equal to 211.0 mg/kg bw per day, on the basis of decreased body weights and body-weight gains in males and females. The NOAEL was 200 ppm, equal to 20.mg/kg bw per day (Milburn, 1992, 1997).

Dogs

In a 90-day study of toxicity, groups of four male and four female beagle dogs were given capsules containing azoxystrobin (purity, 96.2%) at a dose of 0, 10, 50, or 250 mg/kg bw per day for 92 or 93 days. Equal numbers of dogs in each group were treated for each number of days. The dogs were inspected twice per day for clinical or behavioural abnormalities. A detailed physical examination was performed before the start of treatment and at termination. Eyes were examined by indirect ophthalmoscopy at week 1 and before termination. Body weight and food consumption were measured weekly. Blood for measurement of haematological and clinical chemistry parameters

was collected from all dogs before the test, and after 4, 8 and 13 weeks of treatment. Urine analysis was performed on all dogs at termination. At the end of the study, a complete gross post mortem was done. The adrenals, brain, kidneys, liver, epididymides, testes and thyroid glands were weighed. The organs specified were examined microscopically.

No dogs died during the study. Treatment-related clinical observations in males and females included increases in salivation at dosing and increased incidence of salivation, fluid faeces, vomiting, and regurgitation primarily in dogs at 250 mg/kg bw per day (statistical analysis was not performed). All males and three of the females at 250 mg/kg bw per day exhibited salivation and/or salivation at dosing. Isolated occurrences of salivation/salivation at dosing were seen in one female at 50 mg/kg bw per day and one male in each group at 50 and 10 mg/kg bw per day. The incidence of salivation and gastrointestinal findings at 10 mg/kg bw per day was minimal. There was a dose-related increase in the incidence of fluid faeces, which was prominent in dogs at 250 mg/kg bw per day. Minor increases were seen in the incidence of regurgitation and vomiting in males and females at 250 mg/kg bw per day. The Meeting considered that these clinical signs were treatment-related but not relevant for the identification of a NOAEL, being judged to be secondary to local gastrointestinal irritation/disturbances and bolus dosing (capsule).

The weekly body weights of males and females differed statistically significantly from those of dogs in the control group for most weeks at 250 mg/kg bw per day and in females at 50 mg/kg bw per day, although values were within 9% of controls ($p \leq 0.05$ or 0.01). Total body-weight gains were 34% and 38% lower than those of dogs in the control groups for males and females, respectively, at the highest dose. Haematological alterations in one or both sexes at 250 mg/kg bw per day were small, sporadic compared with values for concurrent controls and/or pre-treatment values and not toxicologically relevant. Clinical chemistry parameters that were altered statistically significantly for one or more weeks in males and females at the highest dose compared with those in dogs in the control group included plasma cholesterol (13–26% increase), triglycerides (42–89% increase), alkaline phosphatase activity (24–87% increase), and plasma albumin (7.9–11.6% decrease). Cholesterol was increased in males at the intermediate and lowest dose (17–25%). These results were accompanied by increased absolute liver weight in females at the intermediate and lowest dose (6.3% and 9.3%, respectively), and are consistent with an adverse effect on liver and possibly biliary function. The lack of histopathological correlates and of a clear dose- and time-related response in some cases indicated that the clinical and liver-weight changes were an adaptive response in the liver of dogs at the lowest and intermediate doses. Other clinical chemistry changes did not appear to be treatment-related (plasma sodium, creatinine, and total protein). There were no treatment-related effects on gross or microscopic pathology, food consumption, ophthalmology, or urine analysis. In the absence of histological changes, the increased thyroid weight found in females at the highest dose (37%) was of uncertain toxicological significance.

The LOAEL was 250 mg/kg bw per day on the basis of treatment-related changes in clinical chemistry parameter (cholesterol, triglycerides and alkaline phosphatase) associated with increases in absolute liver weights and decreases in body weights and body-weight gains in males and females. The NOAEL was 50 mg/kg bw per day. The study author identified a NOAEL of 10 mg/kg bw per day, probably on the basis of gastrointestinal findings seen at the next higher dose and above (Allen, 1993; 1995a, 1995b).

In a 1-year study of oral toxicity, groups of four male and four female beagle dogs were given capsules containing azoxystrobin (purity, 96.2%) at a dose of 0, 3, 25, or 200 mg/kg bw per day for 52 weeks. The dogs were inspected twice per day for morbidity or mortality, with clinical signs being checked daily. Thorough examinations were given weekly. Body weights were recorded weekly and food consumption was measured daily. Eyes were examined by indirect ophthalmoscopy at weeks

13, 26, 39 and before termination. Clinical examinations, including cardiac and pulmonary auscultation, were conducted at weeks 13, 26, 39, and before termination. Blood was collected from all dogs before the test, and during weeks 4, 13, 26 and 52 for measurement of haematological and clinical parameters. Urine analysis was performed on all dogs before the test, at week 26 and at termination. At the end of the study, a complete gross post mortem was done. The adrenals, brain, epididymides, kidneys, liver, thyroid and parathyroid, and testes/ovaries were weighed. The organs specified were examined microscopically.

No dogs died before the scheduled termination date. There were no effects on body weight or food consumption related to the administration of azoxystrobin. There were no treatment-related findings noted at the veterinary or ophthalmic examinations. The most notable treatment-related clinical observation was an increase in the incidence of fluid faeces in males and females at 200 mg/kg bw per day: there were 414 occurrences in 4 out of 4 males and 115 occurrences in 4 out of 4 females compared with 3 occurrences in 2 out of 4 males and 6 occurrences in 2 out of 4 females in the control group (statistical analysis was not performed). Females at the highest dose had minor increases in salivation (21 occurrences in 3 out of 4 females at the highest dose compared with 0 out of 4 in the control group) and salivation at dosing (80 occurrences in 3 out of 4 females at the highest dose, compared with 0 out of 4in the control group), although the combined frequency was similar to that of males in the concurrent control group. The Meeting considered that these clinical signs were treatment-related, but not relevant for the identification of a NOAEL, being judged to be secondary effects attributable to local gastrointestinal irritation/disturbances and bolus dosing (capsule).

Minor changes in MCV, MCH, prothrombin time, leukocyte count, neutrophils, kaolin-cephalin time, platelets, lymphocytes, and monocytes were observed; however, these changes in haematological parameters were not considered to be toxicologically significant, being small in magnitude, without a dose–response relationship and transient in nature. Treatment-related clinical chemistry changes at 200 mg/kg bw per day ($p \leq 0.05$ or 0.01) for one or more weeks included increased concentrations of plasma cholesterol (males and females, 14–48%), triglycerides (males and females, 65–124%,), alkaline phosphatase activity (males and females, 17–156%), gamma-glutamyl transferase activity (females, 74–112%) and lowered plasma albumin (males, 9.4–13%). Males at the intermediate dose had increased concentrations of cholesterol (23–27%) and triglycerides (65%). These results suggest that azoxystrobin has an effect on liver and possibly biliary function. Minor and/or transient alterations ($p \leq 0.05$ or 0.01) in total plasma protein, bilirubin, calcium, phosphorus, urea, potassium, and sodium concentrations were observed in one or both sexes. Clinical chemistry changes observed in males and females lacked histopathological correlates There was a small decrease in absolute brain weight in males at the highest dose (6.5%, $p \leq 0.05$) that is of uncertain biological significance. This small decrease in absolute brain weight was of unknown etiology and uncertain biological significance: it was not correlated with any histopathological findings, and the relative-to-body brain weight was not clearly affected. There was a dose-related increase in liver weight in males at the highest dose (15%, $p \leq 0.01$) and in females at the intermediate (12%, $p \leq 0.05$) and highest dose (19%, $p \leq 0.01$). Increases in liver weights seen in females at the intermediate dose were considered to be an adaptive response because liver-enzyme parameters were not affected and no liver pathology was seen. There were no treatment-related effects on urine analysis and gross or microscopic pathology in male or female dogs.

The NOAEL was 25 mg/kg bw per day on the basis of alterations in clinical chemistry parameters and increases in liver weights seen at the LOAEL of 200 mg/kg bw per day, the highest dose tested. The study author identified the no-observed-effect level (NOEL) as 3 mg/kg bw per day (Allen, 1994), although the sponsor contended that there were no adverse effects were identified in the study (Syngenta, 2007).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity, groups of 55 male and 55 female C57BL/10JfAP/Alpk mice were given diets containing azoxystrobin (purity, 96.2%) at a concentration of 0, 50, 300, or 2000 ppm (equal to 0, 6.2, 37.5, or 272.4 and 0, 8.5, 51.3, or 363.3 mg/kg bw per day for males and females, respectively) for 104 weeks. Additional groups of five males and five females were used as microbiological sentinels and fed either control diet or diet containing azoxystrobin at 2000 ppm. Prepared diets were stored at room temperature. Stability, homogeneity and dietary concentrations were confirmed analytically. The mice were inspected daily for mortality and morbidity. Changes in clinical condition or behaviour were recorded daily. Body weights were measured weekly for the first 12 weeks, then every 2 weeks thereafter and at termination. Food consumption was measured weekly for the first 12 weeks, then every 4 weeks thereafter until termination. Water consumption was not measured. An ophthalmoscopic examination was not done. Blood was collected from 11 males and 11 females per group at weeks 53, 79 and at termination. Differential leukocte counts and erythrocyte morphology were performed on mice in the control group and mice at 2000 ppm. Clinical chemistry and urine analysis were not performed. All mice that died and those that were sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed (adrenals, brain, kidneys, liver and testes/ovaries). Tissues were collected for histological examination.

Azoxystrobin was homogenously distributed in the diet and was stable in the diet for 56 days storage at room temperature. The measured test concentrations were within the range of 10% of the target concentrations except for one diet containing azoxystrobin at 2000 ppm that was 117% of the target concentration.

At the end of the study, the survival of males was 58%, 61%, 60% and 63% (control, lowest, intermediate and highest dose, respectively). The survival of was similar at 47%, 38%, 45% and 56% (control, lowest, intermediate and highest dose, respectively). No effects were observed on mortality, clinical signs, haematology, or gross or microscopic pathology. Mean body weights of males at 2000 ppm were significantly ($p \leq 0.01$) lower (5–12%) than those of mice in the control group from week 2 and continuing until the end of the study. Final body weights of males at the highest dose were 94% those of the controls. Body weights of males at 300 ppm were also statistically significantly lower compared with those of the controls at weeks 2, 3, 35, and 61–83; however, final body weights gave evidence for recovery (101% of mice in the control group). No differences in body weights were observed for males at 50 ppm when compared with mice in the control group. Females at 2000 ppm had significantly ($p \leq 0.01$ at week 8 only, $p \leq 0.05$) lower mean body weights (2–7%) compared with those of the controls from study week 3 and continuing until the end of the study. Final body weights of females at the highest dose were 93% those of females in the control group. Although food consumption was similar in treated and control groups, overall food utilization was significantly ($p \leq 0.01$) lower in males and females at the highest dose during weeks 1–12 (the only interval for which food utilization values were calculated). Absolute kidney weights of males at 2000 ppm were significantly ($p \leq 0.05$) less than those of the controls. Absolute kidney weights of females at 2000 ppm were slightly lower than those of the controls (not significant). No significant differences in adjusted kidney weights (organ weight adjusted for body weight) were observed in males and females at 2000 ppm. Absolute liver weights were not affected at any dose tested. However, adjusted liver weights were increased compared with those of mice in the control group for males (14%) and females (18%) at the highest dose. In the absence of any histopathological findings, the Meeting considered that these changes in liver weights were adaptive. The changes in kidney weights were not considered to be adverse because there were no histological findings in the kidney. No evidence of carcinogenicity was observed at the doses tested.

The LOAEL for systemic toxicity was 2000 ppm, equal to 272.4 mg/kg bw per day, on the basis of reduced body weights in males and females. The NOAEL for systemic toxicity was 300 ppm, equal to 37.5 mg/kg bw per day (Moxon, 1995a).

Rats

In a combined long-term study of toxicity and carcinogenicity, groups of 52 male and 52 female Alpk:APfSD rats were given diets containing azoxystrobin, (purity, 96.2%) at a concentration of 0, 60, 300 or 750 ppm/1500 ppm (males/females), equal to 0, 3.6, 18.2, and 34.0 mg/kg bw per day for males and 0, 4.5, 22.3, and 117.1 mg/kg bw per day for females, for 104 weeks. An additional 12 males and 12 females per group were designated for interim sacrifice at week 52. Owing to excessive mortality, the highest dose was reduced to 750 ppm, equal to 34 mg/kg bw per day, in males from week 52 and the rats in this group designated for interim sacrifice were retained with the main study. Additional groups of seven males and seven females were used as microbiological sentinels and fed either control diet or diet containing azoxystrobin at 1500/750 ppm. Diets were prepared in batches of 30 or 60 kg throughout the study. Stability, homogeneity and dietary concentrations were confirmed analytically. the rats were inspected twice per day for mortality and morbidity. Changes in clinical condition and behaviour were recorded daily. Detailed clinical observations were recorded weekly. Body weights were measured weekly for the first 14 weeks, then every 2 weeks for the rest of the study. Food consumption was measured for the first 14 weeks, at week 16, and every fourth week thereafter. Water consumption was not measured. An ophthalmoscopic examination was performed before treatment and at week 54. The eyes of rats in the control group and the group at the highest dose were examined before termination. Blood was collected at weeks 14, 27, 53, 78 and week 105 of treatment. Urine was collected at weeks 13, 26, 52, 78 and 104. Haematological parameters examined were erythrocyte count, leukocyte count, haematocrit (erythrocyte volume fraction), haemoglobin concentration, platelet count, differential leukocyte count and cell morphology. Standard clinical chemistry and urine analysis parameters were examined. At weeks 53 or 105, the designated rats were necropsied and examined histopathologically. Liver, kidney, brain, testes, ovaries, and adrenals were removed and weighed. All tissues were examined histologically, except oral and nasal cavities, which were stored. The common bile duct and intraduodenal bile duct were taken from all rats with bile-duct distension from approximately week 39 and from all rats killed or found dead from week 53.

Azoxystrobin was uniformly distributed in the diet and was stable at room temperature for 66 days. The measured test concentrations ranged from 91.2% to 110.7% of the nominal values. Overall mean achieved dietary concentrations were within ± 2.0% of the nominal concentrations.

Distended abdomens were observed in males starting from week 17, with 5, 0, 5, and 15 rats affected in the control group, and at 60, 300, and 1500/750 ppm, respectively. Hunched posture was observed in males in a dose-related manner, with 3, 11, 12, and 17 rats affected, respectively. There was an apparent increased incidence of opaque eyes in males (0, 4, 2, and 5 rats in the control group, and at 60, 300 and 1500/750 ppm, respectively). No treatment-related clinical signs were observed in females at any dose.

By week 52, survival rates of the males receiving the diets containing azoxystrobin at 0, 60, 300, or 1500 ppm were 97%, 100%, 98%, and 86%, respectively, prompting the dose reduction for the group receiving the highest dietary concentration. Survival rates at week 104 for the control group, and at the lowest, intermediate and highest dose were 37%, 38%, 29%, and 30%, respectively, for males and 45%, 62%, 62%, and 68%, respectively, for females. The lower survival rate for females in the control group did not develop until after week 100.

Males at the highest dose had statistically significantly lower body weights (92–95%) compared with those of males in the control group beginning at week 2 and continuing until week 101 (except for week 87, when no difference occurred). Females at the highest dose had statistically significantly lower body weights (87–94%) than the controls beginning at week 2 and continuing until

study termination. Food consumption was significantly lower (95%) in males at the highest dose at weeks 1–20, 48, and 96 when compared that for controls. Food consumption for females at the highest dose was significantly less (91–96%) than that of females in the control group at weeks 1, 3–11, 13–36, 44, 56, and 68. Food utilization was significantly ($p \leq 0.01$) reduced in males at the highest dose for each of the intervals calculated: weeks 1–4, 5–8, 9–12, and 1–12. Females at the highest dose had significantly ($p \leq 0.01$) reduced food utilization compared with that of controls for weeks 1–4 and 1–12.

Several haematological parameters for rats at the intermediate and highest dose were occasionally statistically significantly different than the values for rats in the control group, but no dose- or treatment-related pattern was observed. Reduction in the activity of alkaline phosphatase, plasma alanine aminotransferase and aspartate aminotransferase was observed at various time-points and doses. These changes were considered not to be toxicologically relevant since they were small in magnitude and lacked any clear dose–response relationship.

Several urinary parameters for the treated groups were occasionally significantly different from the values for controls, but there were no dose- or treatment-related trends apparent for males or females. At weeks 52–54 there was a dose-related increase in the number of males with minute lens opacity, with 1, 2, 5, and 7 rats affected in the groups at 0, 60, 300, and 1500 ppm, respectively. No treatment-related ophthalmoscopic findings were observed in females at weeks 52–54 or in males or females at weeks 103–104. Adrenal weights were statistically significantly lower than those of controls in females at 1500 ppm at week 53. At terminal sacrifice, males and females at the highest dose had significantly lower adrenal gland weights (84% of values for controls) and kidney weights (83% and 89% of values for controls, respectively) compared with controls. Absolute liver weights were increased in females at the highest dose at week 53, but not at terminal sacrifice. In the common bile duct of males at the highest dose, there were significant increases ($p \leq 0.01$) in the rates of distension (13 out of 47), cholangitis (13 out of 47), thickening of the wall (11 out of 47), and epithelial hyperplasia (9 out of 47); these lesions were not observed in rats in the control group (0 out of 34) or males and females in any other group receiving azoxystrobin.

There was no evidence of carcinogenic activity in this study. Among female rats, there was a significant dose-related decrease in the incidence of benign fibroadenomas of the mammary gland with 10 out of 52, 3 out of 52, 2 out of 52 ($p \leq 0.05$), and 1 out of 52 ($p \leq 0.01$) affected in the control group, and at 60, 300, and 1500 ppm, respectively.

The NOAEL was 300 ppm, equal to 18.2 mg/kg bw per day, on the basis of reduced body weights, food consumption and food efficiency, and bile-duct lesions (males only) seen at the LOAEL of 750 ppm, the highest dose tested (equal to 34.0 mg/kg bw per day). The study author identified a NOEL of 300 ppm, equal to 18.2 mg/kg bw per day (Milburn, 1995).

2.4 Genotoxicity

Azoxystrobin gave mixed responses in a battery of assays for genotoxicity. Negative results were obtained in the Ames test, and tests for unscheduled DNA synthesis (UDS) and for micronucleus formation in vivo. Azoxystrobin gave a weak positive response in two studies in mammalian cells (mouse lymphoma cells and human lymphocytes). The latter findings suggest that azoxystrobin has a clastogenic potential in vitro since the increased occurrence of small colonies in the mouse lymphoma cell assay is considered to be indicative of chromosome aberrations rather than of point mutations. However, azoxystrobin has shown to give negative results in assays for chromosomal damage (i.e. clastogenicity) in vivo and for general DNA damage at high doses of 2000 mg/kg bw or above. The Meeting concluded, therefore, that the clastogenic effects seen in vitro are not expressed in the whole animal. Furthermore, long-term studies have not shown any evidence of carcinogenicity

in mice or rats. Based on the overall weight of evidence, the Meeting concluded that azoxystrobin is unlikely to be genotoxic.

2.5 *Reproductive toxicity*

(a) Multigeneration studies

Rats

In a two-generation study of reproductive toxicity, groups of 26 male and 26 female Alpk:APfSD (Wistar-derived) rats were given diets containing azoxystrobin (purity, 96.2%) at a concentration of 0, 60, 300, or 1500 ppm. The average achieved intake of azoxystrobin during the premating interval for the F_0 and F_1 generations was as follows: 0 6.4, 32.3, or 165.4 mg/kg bw for males and 0, 6.8, 33.8, or 175.0 mg/kg bw per day for females. All rats were mated on a 1 : 1 ratio. All rats were exposed continuously to diets containing the test material throughout the study. Diets were prepared in batches of 60 kg and stored at room temperature. Stability, homogeneity and dietary concentrations were confirmed analytically. The rats were inspected daily for clinical observations, mortality, and morbidity. Physical examinations were performed weekly. Body weights were recorded weekly during the mating period. Females were weighed on days 1, 8, 15, and 22 of gestation, and days 1, 5, 11, 16, 22 and 29 of lactation. Food consumption was measured weekly throughout the mating

Table 5. Results of studies of genotoxicity with azoxystrobin

End-point	Test system	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation[a] (Ames test)	*S. typhimurium* strains TA98, TA100, TA1535 and TA1537 *E. coli* WP2P, WP2P *uvrA*	100–5000 µg/plate ±S9, in DMSO	97.2	Negative	Callander (1992)
Forward mutation[b]	Mouse lymphoma L5178Y cells	8–60 µg/ml (test 1) 34–80 µg/ml (test 2) 26–80 µg/ml (test 3) ±S9, in DMSO	96.2	Weakly positive	Callander & Clay (1993); Fox & Callander (1995)
Chromosomal aberration[b]	Human lymphocytes	1–20 µg/ml (–S9) 25–200 µg/ml (+S9)	95.2	Weakly positive	Fox & Mackay (1992, 1995a)
In vivo					
Micronucleus formation[c]	C57 BL/6JfBL10/AlpK mice (male and female)	5000 mg/kg bw (single oral dose)	97.2	Negative	Jones & Mckay (1992); Fox & Mackay (1995b)
Unscheduled DNA synthesis	Hepatocytes from male Aldereley Park (Alpk:APfSD) rats	1250 and 2000 mg/kg bw (single oral dose)	97.2	Negative	Lane & Kennelly (1992); Fox & Mackay (1995c)

S9, 9000 × *g* supernatant from livers of male rats.

[a] Precipitation at concentrations of 2500 and 5000 µg/plate.

[b] Higher concentrations were limited by cytotoxicity.

[c] Groups of five males and five females per dose. Azoxystrobin administered via gavage in corn oil. Bone marrow collected at 24 h and 48 h. Clinical signs after dosing included tiptoe gait, piloerection, diarrhoea and urinary incontinence on day of dosing.

period and for females during gestation and lactation. Estrous cycles were monitored with vaginal smears taken during the mating period and until mating was confirmed. The duration of gestation was calculated. Females were allowed to deliver normally and rear young to weaning on day 29. 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 daily for survival. The number, sex and weight of pups were recorded on postnatal days 1, 5, 11, 16, 22, and 29. All parental (F_0) and F_1 rats and those found dead and killed in extremis were necropsied and examined macroscopically. All pups that were not selected for the next generation were killed on postnatal day 29. Selected male and female pups received a full examination post mortem. Selected tissues (liver, uterus, cervix, vagina, ovaries, mammary glands, testes, epididymides, prostate, seminal vesicle and pituitary gland) were examined histopathologically. Liver, epididymides, and testes/ovaries were weighed.

The analytical data indicated that the mixing procedure for the diets was adequate and that the variation between nominal and actual dietary concentrations received was within 10% of the nominal values.

There were no treatment-related clinical signs of toxicity or increases in mortality noted at any dose. However, one F_0 male and one F_1 male from the groups at 1500 ppm were sacrificed in a moribund condition and exhibited treatment-related distention of the common bile duct. At 1500 ppm, systemic toxicity in the F_0 and F_1 adults (males and females) was apparent as reduced adjusted body weights (3–12%, $p \leq 0.01$ or 0.05) and food consumption (5–14%, $p \leq 0.05$ or 0.01) during the pre-mating intervals. At 1500 ppm, gestational body weights were reduced (within 5% of values for controls) for F_0 and F_1 females. In addition, treatment-related increases in liver weights adjusted for final body weights were noted in the F_0 and F_1 males and females (15–38%, $p \leq 0.01$ or 0.05) at 1500 ppm. Treatment-related distention of the common bile duct was also noted in 3 and 11 of the F_0 and F_1 males at 1500 ppm, respectively, on examining grossly. Treatment-related histopathological lesions of the common bile duct in the adult males at the highest dose were characterized as epithelial hyperplasia of the intraduodenal portion, cholangitis, ulceration of the dilated region, and small basophilic deposits in the lumen. Treatment-related increases in severity of proliferative cholangitis were also observed in the livers of the F_0 and F_1 males at 1500 ppm. Males and female in the F_1a and F_2a at 1500 ppm had treatment-related increases in the adjusted (for final body weight) liver weights (10–13%, $p \leq 0.01$).

No treatment-related clinical signs of toxicity were observed in pups of either generation. Treatment-related reductions in adjusted (for initial weight) pup body weights were observed in the F_0a and F_2a pups at 1500 ppm (8–21%, $p \leq 0.05$ or $p \leq 0.01$). Treatment-related increases in the adjusted mean liver weights were noted in the F_1a and F_2a males and females at 1500 ppm (10–13%, $p \leq 0.01$). There were no treatment-related macroscopic or microscopic findings in the F_1a or F_2a litters. None of the reproductive parameters were affected at any of the dietary concentrations tested.

The LOAEL for systemic toxicity was 1500 ppm, equal to165.4 mg/kg bw per day, on the basis of reduced adjusted body weight, feed consumption, feed utilization, an increased in adjusted liver weights and histopathology (males). The NOAEL for systemic toxicity was 300 ppm, equal to 32.3 mg/kg bw per day. The NOAEL for offspring toxicity was 300 ppm, equal to 32.3 mg/kg bw per day, on the basis of reduced pup body weight and increased liver weights seen at the LOAEL of 1500 ppm, equal to 165.4 mg/kg bw per day. The NOAEL for reproductive toxicity was ≥ 1500 ppm, equal to ≥ 165.4 mg/kg bw per day, the highest dose tested (Moxon, 1994b, 1995b, 1997).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 24 female Alpk:APfSD rats were given azoxystrobin (purity, 95.2%) at a dose of 0, 25, 100 or 300 mg/kg bw per day by gavage in corn oil (dosing volume, 1 ml/100 g bw) from day 7 to 16 of gestation, inclusive. Stability, homogeneity and dose

concentrations were confirmed analytically. All rats were observed daily for clinical signs of toxicity, mortality and moribundity. Maternal body weights were recorded on days 1, 4, 7–16, 19 and 22 of gestation. Food consumption was determined at 3-day intervals until day 22 of gestation. On day 22 of gestation, all surviving dams were sacrificed and subjected to gross necropsy. Examinations at sacrifice comprised uterine weight, number and positions of implantations; corpora lutea in each ovary, individual fetal weights, percentage preimplantation loss, percentage postimplantation loss, early and late intrauterine deaths. All fetuses were weighed, sexed, and examined for external malformations/variations. Each fetus was examined viscerally by fresh dissection and the sex was verified. The fetuses were then eviscerated and fixed in 70% industrial methylated spirits. After approximately 24 h, the brain was examined for macroscopic abnormalities and the carcasses were stained with Alizarin Red S for evaluation of the skeleton.

Analysis of the dosing solution indicated that the test material was homogenously distributed and was stable for 25 days. The achieved concentrations were within 4% of the nominal values.

Three rats at 300 mg/kg bw per day were found dead after receiving two daily doses and one rat was killed in extremis. Severe signs of toxicity were observed in another 12 rats. Dosing of the remaining rats in this group was suspended. These rats were able to recover and continue to scheduled termination. The remaining 12 rats at the highest dose did not start treatment and no assessment of developmental toxicity was made at this dose. Gross necropsy of rats at the highest dose that were found dead revealed red areas and thin walls in the stomach or jejunum. In the groups at the intermediate dose, two animals showed haemorrhagic areas in the stomach at terminal necropsy; these findings were considered to be likely to be related to local irritation caused by the administration of azoxystrobin by gavage.

At 100 mg/kg bw per day, minimally reduced body weights (< 2%) were observed ($p < 0.05$), although body-weight gain and food consumption were not affected. Clinical signs during dosing included diarrhoea (42%), urinary incontinence (17%) and salivation between days 9 and 16 (71%). At 25 mg/kg/bw per day, salivation was observed in 29% of rats between days 11 and 16. The Meeting considered that these clinical signs were treatment-related but not relevant for the identification of a NOAEL, being considered to be secondary effects caused by local gastrointestinal irritation/disturbances and bolus dosing by gavage in corn oil.

In the conceptus, no significant adverse developmental effects were observed. General reduced ossification was seen at all doses, the incidence of which was not statistically different between the controls group and groups receiving azoxystrobin. There was, however, a statistical increase (6.9% vs 2.6%, $p < 0.05$) in the rats with a PES score of 6 at 100 mg/kg bw per day vs controls. Since this possible minimal increase in reduced ossification was not supported by increases in other related end-points, and was not statistically different from controls, the Meeting considered that it was not of toxicological significance.

The NOAEL for maternal and developmental toxicity was 100 mg/kg bw per day, the highest dose tested. The study author concluded that the NOAEL for maternal and developmental toxicity was 25 mg/kg bw per day, considering that minimal reduction in ossification was a treatment-related effect (Moxon, 1994a).

Rabbits

In a study of developmental toxicity, groups of 20 pregnant New Zealand white rabbits were given azoxystrobin (purity, 96.2%) at a dose of 0, 7.5, 20, or 50 mg/kg bw per day by gavage in corn oil (dosing volume, 2 ml/kg bw) on days 8–20 of gestation, inclusive. Test substance formulations were prepared daily. Stability, homogeneity and dose concentrations were confirmed analytically. All rabbits were observed twice per day for mortality or clinical signs of toxicity. Maternal body weights were recorded on days 0 and 4 of gestation and daily on days 8–20, and on days 23, 26 and 30 of gestation. Food consumption was measured on days 8, 11, 14, 17, 20, 23 and 26 of gestation. On

day 30 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 nonviable fetuses, early and late resorptions, and the total number of implantations were recorded. All fetuses were weighed and examined for external malformations/variations. Each fetus was examined viscerally by fresh dissection and the sex determined. The brain from each fetus was examined by mid-coronal slice. All carcasses were eviscerated and processed for skeletal examination.

The analytical data indicated that the mixing procedure for the dosing solution was adequate and that the variation between nominal and actual doses received by the rabbits was acceptable (within 3% of nominal values).

There were two, four, three and seven deaths in the control group and at 7.5, 20 and 50 mg/kg bw per day, respectively. One rabbit at 50 mg/kg bw per day was found dead; the other deaths resulted from premature termination after abortion or deterioration in clinical condition, usually between days 12 and 20 of gestation. The following clinical observations were reported: blood on tray, sporadic in two, one, two, six rabbits in the control group and at 7.5, 20 and 50 mg/kg bw per day, respectively; general coat staining, two, two, three, five rabbits in the control group and at 7.5, 20 and 50 mg/kg bw per day, respectively; diarrhoea, two, four, seven, seven rabbits in the control group and at 7.5, 20 and 50 mg/kg bw per day, respectively. While these occurrences were observed in a dose-related manner, their toxicological significance is uncertain. At the start of dosing, slight loss of body weight for all groups including the controls was observed. The loss in body weight was more severe at 20 and 50 mg/kg bw per day than in the control group. Five rabbits at 50 mg/kg bw per day showed progressive bosy-weight loss from which they did not recover and were therefore killed. However, two, three and two rabbits were killed for similar reasons in the control group and at 7.5 and 20 mg/kg bw per day, respectively. Food consumption data were inconclusive owing to food wastage and other factors. Some rabbits showed very little food consumption. The number of rabbits showing negligible food consumption during the dosing period was five, six, eight and nine in the control group and at the lowest, intermediate and highest dose, respectively. No dose-related adverse effects were noted during necropsy, either in rabbits that died during the study, or in rabbits sacrificed at the end of the study.

The incidence of fetuses with major defects was 8, 0, 2 and 13 in the control group and at 7.5, 20 and 50 mg/kg bw per day, respectively. At the highest dose, nine fetuses (8.6%) from two litters (16.7%) had open eye, the majority being bilateral. One fetus at the highest dose had cleft palate. Other effects were of low occurrence, were not dose-related, and were not associated with treatment. These effects included internal hydrocephaly, encephalocoele, fenestration in parietal, reduced pulmonary artery, enlarged aorta. Many of these effects occurred in the control group only. Fused sternebrae (third and fourth, or fourth and fifth) was noted in the group at the highest dose. The occurrence was statistically ($p < 0.01$) above control values and involved twelve fetuses from four litters. Most of the affected fetuses were from the litters with open eye. While these changes were considered to be compound-related, their biological significance was uncertain.

Owing to excessive food wastage, maternal death and other unidentified factors, the Meeting did not consider that the results of this study were appropriate for the identification of an acceptable daily intake (ADI) or an acute reference dose (ARfD).

The NOAEL for maternal toxicity was 7.5 mg/kg bw per day on the basis of decreased body weights, clinical signs of toxicity and marked reduction in food consumption seen at the LOAEL of 20 mg/kg bw per day and above. The NOAEL for developmental toxicity was 20 mg/kg bw per day on the basis of fused sternebrae, open eyes and cleft palates seen at 50 mg/kg bw per day (Moxon, 1994c).

In a second study of developmental toxicity, groups of 21 female New Zealand White rabbits were given azoxystrobin (purity, 96.2%) at a dose of 0, 50, 150 or 500 mg/kg bw per day by gavage in corn oil (dosing volume, 1 ml/kg bw) from days 8 to 20 of gestation, inclusive. Rabbits in the control

group received the appropriate volume of corn oil only. All other experimental details were similar to those for the study of developmental toxicity by Moxon (1994c, described above).

The analytical data indicated that the mixing procedure for was adequate and that the variation between nominal and actual doses given to the rabbits was acceptable (within 3% of nominal values). None of the intercurrent deaths in the study was considered to be associated with the administration of azoxystrobin. The occurrence of intercurrent deaths was one, two, one, and two in the control group and at 50, 150 and 500 mg/kg bw per day, respectively. One rabbit at 500 mg/kg bw per day was killed on day 11 and was found to have an intussusception of the colon and severe body-weight loss. This death was not considered to be treatment-related. Two rabbits in the group at 150 mg/kg bw per day were killed; one was killed on day 21 of gestation after abortion and other rabbit on day 17 of gestation because of excessive body-weight loss starting from day 8 of gestation.

Clinical signs included diarrhoea and/or staining in the genital area in 1, 7, 15, and 18 rabbits in the control group and at 50, 150 and 500 mg/kg bw per day, respectively, beginning generally around days 9 and 10 of gestation. No other treatment-related signs were observed. The Meeting considered that these clinical signs were treatment-related but not relevant for the identification of a NOAEL, being considered to be secondary effects caused by local gastrointestinal irritation/disturbances and a bolus dosing by gavage in corn oil.

At 150 mg/kg bw per day and 500 mg/kg bw per day, significant ($p < 0.01$) but transient reductions (–33%, –51%, respectively) in food consumption were observed during the first 3 days of dosing. At 500 mg/kg bw per day, decreased body-weight gain (–45%) was observed during the dosing period. Slight reductions in body weights were observed on days 9–12 at 50 and 150 mg/kg bw per day; however, the decrease in body weights did not occur in a dose-related manner. No treatment-related increases in gross lesions were observed.

No dose-related or statistically significant increases in external or visceral anomalies were observed. Although skeletal anomalies, mainly variations, were common in all groups (including concurrent controls), there were no statistically significant increases, nor were there any trends in dose–response observed. The LOAEL for developmental toxicity was > 500 mg/kg bw per day. The NOAEL for developmental toxicity was 500 mg/kg bw per day.

The NOAEL for maternal toxicity was 150 mg/kg bw per day on the basis of decreased body-weight gain seen at the LOAEL of 500 mg/kg bw per day. The NOAEL for developmental toxicity was 500 mg/kg bw per day, the highest dose tested. The study author identified the NOAEL for maternal toxicity as 50 mg/kg bw per day owing to marginal decreases in body weight, diarrhoea and food consumption at 150 mg/kg bw per day (Moxon, 1995c).

A technical review by Lewis (1995) evaluated the results of the two studies of developmental toxicity in rabbits, described above. Lewis (1995) cites several studies in pregnant and non-pregnant rabbits carried out to determine a suitable vehicle for the administration of azoxystrobin and also the appropriate volume of the vehicle, and concludes that the results of the first study of developmental toxicity (Mowon, 1994) in rabbits given azoxystrobin at a dose of 50 mg/kg bw per day were invalid owing to difficulty in selecting appropriate doses with respect to acceptable maternal toxicity, lack of any clear dose–response relationship, and variability in response within the experimental groups. This review also states that the evaluation of these data suggested that the dosing vehicle, corn oil, used at 2 mg/kg bw may have contributed to the pattern of observed effects.

2.6 Special studies

(a) Acute neurotoxicity:

In a study of acute neurotoxicity, three groups of 10 male and 10 female Alpk:ApfSD rats were given a single dose of azoxystrobin (purity, 96.2%) at 0, 200, 600 or 2000 mg/kg bw by gavage in corn

oil (dosing volume, 10 ml/kg bw) and observed for the following 14 days. All the rats were evaluated in functional observational battery (FOB) and motor activity tests on days –7, 1 (2 h after dosing; time of peak effects), 8 and 15. All rats were observed before the study start and daily throughout the study for any changes in clinical condition. Body weights and food consumption were measured weekly throughout the study. Five males and females in the control group and at the highest dose were perfused in situ and evaluated for microscopic neuropathology.

Measurements of dosing solutions indicated that dosing solutions were homogeneous, were stable for at least 8 days and that the rats received appropriate doses.

At doses of 200 mg/kg and higher, diarrhoea/signs of diarrhoea were observed at 2 h after dosing in males and females. Tip-toe gait and hunched posture at 2 h were also observed in rats receiving azoxystrobin but not in rats in the control group (no dose–response relationship observed). No apparent dose-related increase in incidence of clinical signs was evident. Recovery from all of these findings was usually apparent by day 2. The Meeting considered that the diarrhoea was treatment-related but not relevant for the identification of a NOAEL, this effect being considered as a secondary effect caused by local gastrointestinal irritation/disturbances. No treatment-related effects on survival, food consumption, motor activity, brain weight/dimensions, or gross/microscopic pathology were observed. Body weights of males at 2000 mg/kg bw were slightly decreased (2.9% and 2.6% at day 8 and 15). Statistically significantly decreased body weight in the males at 200 mg/kg bw was observed on day 15. However, a dose–response relationship was not evident and the decrease was considered to be incidental. There was no effect on the amount of food consumed in males or females at any dose.

Statistically significant increases in landing foot splay on day 8 in females at 600 and 2000 mg/kg bw were noted (23.7% and 20.5% higher than controls, respectively; on day 1, females at 600 and 2000 mg/kg bw had non-statistically significantly increased values of 11.8% and 12.5%, respectively). The findings of hunched posture, tip-toe gait and increased landing-foot splay were not considered indicative of neurotoxicity owing to the lack of any effect on the day of dosing (only marginal non-significant increase seen) and to lack of a clear dose–response relationship and the association with marked gastrointestinal disturbance. There were no effects on brain weight, length or width. There were no treatment-related macroscopic or histopathological changes, including in the nervous system.

The NOAEL for systemic toxicity was 2000 mg/kg bw. No LOAEL was identified. The NOAEL for neurotoxicity was ≥ 2000 mg/kg bw (Horner, 1994 and 1996).

(b) Short-term study of neurotoxicity:

In a short-term study of neurotoxicity, groups of 12 male and 12 femaleAlpk:APfSD rats were given diets containing azoxystrobin (purity, 96.2%) at a concentration of 0, 100, 500 or 2000 ppm (equal to 0, 8.0, 38.5 or 161 mg/kg bw per day in males and 0, 9.1, 47.9 or 201.5 mg/kg bw per day in females) for 13 weeks. All rats were evaluated in FOB and motor activity tests in weeks –1, 5, 9 and 14. All rats were observed before the study start and daily throughout the study for any changes in clinical condition. Body weights and food consumption were measured weekly throughout the study. Six male and six female rats from the control group and at the highest dose were perfused in situ and evaluated for microscopic neuropathology.

Analysis of the dietary preparations showed that diets were stable at room temperature for 56 days and the test article was homogeneously distributed in the diet. The overall mean dietary concentrations were within 8% of the nominal values.

There were no deaths or treatment-related changes in clinical condition observed during the study. At 2000 ppm, mean body weights of males were statistically significantly decreased throughout the study (at week 13, 12.6% less than controls). Mean body weights of females were slightly decreased (at week 13, 5.1% less than controls; significant only at week 2). Cumulative body-weight

gains were 18% lower (males) and 10% lower (females) than those of the controls. Food consumption was statistically significantly lower than those of the controls in males (5.4% to 15.4%) but not females. Food utilization in males at 2000 ppm was statistically significantly decreased during weeks 1–4 (9.7%) and 1–13 (11.7%) and was non-significantly less in females during the same periods (11.8% and 14.4%, respectively).

There were no consistent indications of treatment-related neurotoxicity (clinical signs, qualitative or quantitative neurobehavioral effects, brain weight/ dimensions, or gross/microscopic pathology). Statistically significant decreases in landing foot splay in males (week 5: 19%, 16.4% and 24.1%, for the lowest to the highest dose, respectively; week 9, 18% at the highest dose), forelimb grip strength (males: week 5, 14.3%, 14.3% and 19%, for the lowest to the highest dose, respectively; and females, week 14, 12.9%, highest dose), hind-limb grip strength in males (week 5, 13.3%, 15.3% and 12.9%, for the lowest to the highest dose, respectively) and motor activity in females (21%, week 9) weer noted but not considered to be treatment-related owing to lack of a dose–response relationship, inconsistency of observations at different time-points, variability of pre-treatment values and/or small magnitude of response. Brain weight and length were unaffected by treatment. There were no macroscopic or treatment-related microscopic findings at the end of the study.

The LOAEL for systemic toxicity was 2000 ppm, equal to 161 mg/kg bw per day, on the basis of decreased body weight/body-weight gain and food utilization in males and females (marginal in females). The NOAEL was 500 ppm, equal to 38.5 mg/kg bw per day. The NOAEL for neurotoxicity was ≥ 2000 ppm, equal to ≥ 161 mg/kg bw per day (Rattray, 1994, 1996).

(c) Studies on metabolites

No studies on metabolites were submitted by the sponsor.

3. Observations in humans

No observations in humans were provided by the sponsor.

Comments

Biochemical aspects

In an autoradiography study in rats, groups of one male and one female were given azoxystrobin labelled with ^{14}C in either the cyanophenyl, pyrimidinyl or phenylacrylate ring as a single dose at 1 mg/kg bw by gavage. The results of this study indicated that the position of the radiolabel had no significant effect on the rates and routes of excretion or tissue distribution of azoxystrobin, therefore, further metabolism studies were conducted using azoxystrobin labelled in the pyrimidinyl position. In studies in rats given a single oral dose of radiolabelled azoxystrobin, 73–89% of the administered dose was recovered in the faeces and 9–18% in the urine (1 and 100 mg/kg bw) after 7 days. The extent of oral absorption at 1 mg/kg bw was nearly complete since no parent compound was found in the excreta. At least 74–81% of the administered dose was absorbed at 100 mg/kg bw, based on recoveries of radioactivity in the bile and urine. Between 82% and 96% of the administered dose was excreted within the first 48 h. Regardless of the dose administered, residues remaining in the carcass (including organs and tissues) were between 0.31% and 0.62% of the administered dose after 7 days. The highest concentrations were found in the liver (0.009–0.72 µg equivalents/g) and in the kidneys (0.023–1.12 µg equivalents/g) at 7 days. No significant quantities of radiolabel were detected in exhaled air. In a study of biliary excretion, about 57–74% of the administered dose was recovered in the bile within 48 h after administration of a single dose at 100 mg/kg bw by gavage. No parent compound was detected in the bile.

Systemically absorbed azoxystrobin was extensively metabolized. The mass balance for the metabolism study indicated that a substantial percentage (45.6–73.6%) of the radiolabel was unextracted, although the excretion studies showed a total recovery of 91.8–104%, with 72.6–89.3% being in the faeces. Fifteen metabolites were identified in the excreta and seven additional metabolites were detected but not identified (< 4.9% of the administered dose). The major metabolites of azoxystrobin in the bile, urine and faeces resulted from hydrolysis followed by glucuronide conjugation. Azoxystrobin was also hydroxylated at the 8 and 10 positions on the cyanophenyl ring, followed by glucuronide conjugation. A minor pathway involving the cleavage of the ether linkage was identified. Approximately 15–32% of the unchanged azoxystrobin was detected in the faeces of bile-duct cannulated rats and rats at the highest dose. Absorption, distribution, excretion and metabolite profiles were essentially similar in males and females, but sex-specific differences in biotransformation were observed, with the number of metabolites produced being greater in females than in males.

Toxicological data

Azoxystrobin has low acute toxicity when administered by the oral, dermal or inhalation routes. The LD_{50} in rats treated orally was > 5000 mg/kg bw. The LD_{50} in rats treated dermally was > 2000 mg/kg bw. The LC_{50} in rats treated by inhalation (nose only) was 0.7 mg/l. Azoxystrobin was slightly irritating to the eyes and skin of rabbits. Azoxystrobin was not a skin sensitizer as determined by the Magnusson & Kligman (maximization) test in guinea-pigs.

In short-term studies in rats and dogs and long-term studies in mice and rats, the major toxicological findings included decreased body weight and body-weight gains, often accompanied by decreased food consumption and utilization. The major target organs in rats were the liver, kidney and bile duct as shown by changes in organ weights, histopathology, and clinical chemistry parameters. Changes in liver weights, often accompanied by changes in clinical chemistry, were also observed in dogs and mice. Kidney-weight changes in mice were not accompanied by any histopathological findings.

In a 90-day dietary study of toxicity in rats, decreased body weights and body-weight gains were seen at 2000 ppm (equal to 221.0 mg/kg bw per day) and 4000 ppm (equal to 443.8 mg/kg bw per day). At 4000 ppm, decreased food consumption, food utilization, changes in clinical chemistry parameters, increased liver and kidney weights, hepatocellular hyperplasia and enlarged lymph nodes, and reduction in total urinary protein were seen in males. The no-observed-adverse-effect level (NOAEL) was 200 ppm, equal to 20.4 mg/kg bw per day.

In a 90-day and a 1-year study in dogs, clinical observations included increased salivation at dosing, and increased incidences of salivation, vomiting, regurgitation and fluid faeces, beginning in week 1 and occurring throughout the study in some cases. These signs were considered to be treatment-related; however, they were not considered to be relevant for identification of a NOAEL for systemic toxicity because these effects were secondary to local gastrointestinal irritation/disturbances and bolus dosing (capsules). In a 90-day study of toxicity in dogs, decreases in body weights were observed in males and females at 250 mg/kg bw per day, the highest dose tested. Changes in liver weights and in clinical chemistry parameters were observed at the intermediate and the highest dose, indicating adverse effects on the liver and possibly on biliary function. The changes in the liver at 50 mg/kg bw per day were small and without histological correlates, therefore, the Meeting considered that they were not toxicologically relevant. In a 90-day study in dogs, the NOAEL was 50 mg/kg bw per day on the basis of alterations in clinical chemistry (cholesterol, triglycerides and alkaline phosphatase activity), and decreases in body weights seen at the LOAEL of 250 mg/kg bw per day, the highest dose tested. Similar findings were observed in a 1-year study of toxicity in dogs but were mainly confined to the highest dose of 200 mg/kg bw per day. In a 1-year study of toxicity in dogs, the NOAEL was 25 mg/kg bw per day on the basis of changes in clinical chemistry and increases in liver weights seen at 200 mg/kg bw per day. The overall NOAEL in dogs was 50 mg/kg bw per day on the basis of the similarity of effects in the two studies in dogs.

The carcinogenic potential of azoxystrobin was studied in mice and rats. In a study of carcinogenicity in mice, reduced body weights were observed at 2000 ppm, equal to 272.4 mg/kg bw per day. The NOAEL was 300 ppm, equal to 37.5 mg/kg bw per day. There were no treatment-related neoplastic findings in the bioassay in mice.

In a long-term combined study of toxicity and carcinogenicity in rats, the highest dose of 1500 ppm, equal to 108.6 mg/kg bw per day, was excessively toxic in males and was reduced to 750 ppm, equal to 34 mg/kg bw per day, after 1 year. Reduced body weights, food consumption, and food-conversion efficiency was observed in males and females at the highest dose tested. In the common bile duct of males at the highest dose only, there were significant increases in the incidences of distension, cholangitis, thickening of the wall, and epithelial hyperplasia. The NOAEL was 300 ppm, equal to 18.2 mg/kg bw per day. There were no treatment-related neoplastic findings in rats.

Azoxystrobin gave a mixed response in a battery of tests for genotoxicity. It gave a weak positive response in two studies in mammalian cells (mouse lymphoma cells and human lymphocytes). The latter findings suggest that azoxystrobin has a clastogenic potential in vitro since the increased occurrence of small colonies observed in the mouse lymphoma-cell assay is considered to be indicative of chromosome aberrations rather than of point mutations. However, azoxystrobin has been shown to give negative results in assays for chromosomal damage in vivo (i.e., clastogenicity) and for general DNA damage at high doses of 2000 mg/kg bw or above. Therefore, the Meeting concluded that the clastogenic effects seen in vitro are not expressed in the whole animal.

The Meeting concluded that azoxystrobin is unlikely to be genotoxic.

In view of the lack of evidence for a genotoxic potential in vivo and the absence of carcinogenicity in rats and mice, the Meeting concluded that azoxystrobin is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, reproductive parameters were not affected at the highest dose tested (1500 ppm, equal to 165.4 mg/kg bw per day). The NOAEL for parental systemic toxicity was 300 ppm, equal to 32.3 mg/kg bw per day, on the basis of reduced adjusted body weight, feed consumption, feed utilization, and an increase in liver weights and the frequency of histopathological findings in the liver (males only). Offspring toxicity was manifested as a decrease in pup body weights, and a decrease in adjusted mean liver weights was observed in pups of both generations at 1500 ppm, equal to 165.4 mg/kg bw per day. The NOAEL for offspring toxicity was 300 ppm, equal to 32.3 mg/kg bw per day.

In a study of developmental toxicity in rats, treatment at the highest dose (300 mg/kg bw per day) was terminated as this dose was toxic; at this dose, three rats died and one was killed in extremis after two doses. Clinical signs of diarrhoea, salivation and urinary incontinence were seen at 25 and/or at 100 mg/kg bw per day. The Meeting considered these effects to be treatment-related but not relevant for the identification of a NOAEL for systemic toxicity, being considered to be secondary to local gastrointestinal irritation/disturbances and dosing by gavage. There were no effects on fetuses at any doses tested. The NOAEL for maternal and developmental toxicity was 100 mg/kg bw per day, the highest dose tested.

Two studies of developmental toxicity in rabbits were available. The results of the first study were considered to be invalid because of the adverse effects of administration of high volumes of corn oil as a vehicle. Several special studies were conducted in pregnant and non-pregnant rabbits to evaluate the influence of the type and volume of vehicle used for administration by gavage. The results of these studies showed that corn oil at volumes greater than 2 ml/kg bw was harmful. The NOAEL for maternal toxicity in rabbits was 150 mg/kg bw per day (identified in the study using the lowest volume of corn oil for dosing) on the basis of decreased body-weight gain seen at the LOAEL of 500 mg/kg bw per day. There were no effects on fetuses. The NOAEL for developmental toxicity in rabbits was 500 mg/kg bw per day, the highest dose tested.

Azoxystrobin was not embryotoxic, fetotoxic or teratogenic at doses of up to 300 and 500 mg/kg bw per day in rats and rabbits, respectively.

The Meeting concluded that azoxystrobin is not teratogenic.

In a study of acute neurotoxicity in rats, no treatment-related effects on motor activity parameters, brain measurements (weight, length and width) or neurohistopathology were observed at doses of up to and including 2000 mg/kg bw. Increased incidences of transient diarrhoea, tip-toe gait, hunched posture and landing-foot splay were observed in all groups receiving azoxystrobin, although these effects were not dose-related. They were considered to be treatment-related but not relevant for identification of a NOAEL for systemic toxicity, being considered to be secondary to local gastrointestinal irritation/disturbances and bolus dosing by gavage. The NOAEL for systemic toxicity was 2000 mg/kg bw, the highest dose tested. In a short-term study of neurotoxicity in rats, no treatment-related changes in mortality, clinical signs, FOB, motor activity, brain measurements (weight, length, and width), gross necropsy, or neurohistopathology were observed at doses of up to 2000 ppm, equal to 161 mg/kg bw per day, the highest dose tested. The NOAEL for systemic toxicity was 500 ppm, equal to 38.5 mg/kg bw per day, on the basis of decreased body weight and body-weight gain and food utilization in males and females seen at the LOAEL of 2000 ppm, equal to 161 mg/kg bw per day.

Azoxystrobin was not considered to be neurotoxic on the basis of the available data.

No significant adverse effects were reported in personnel working in plants producing azoxystrobin.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.2 mg/kg bw based on a NOAEL of 300 ppm (equal to 18.2 mg/kg bw per day) in a 2-year study of carcinogenicity in rats, identified on the basis of reduced body weights, food consumption and food efficiency, and bile-duct lesions seen at 750 ppm (equal to 34 mg/kg bw per day) and above, and using a safety factor of 100.

The Meeting concluded that it was unnecessary to establish an ARfD for azoxystrobin because no toxicity could be attributable to a single exposure in the available database, including a study of developmental toxicity in rats and rabbits and a study of acute neurotoxicity in rats. The mortality seen in the study of developmental toxicity in pregnant rats at 300 mg/kg bw per day was associated with gross local gastrointestinal pathology and was not seen in pregnant rabbits. The Meeting considered that clinical signs observed in dogs and rats were related to local gastrointestinal effects seen after bolus dosing by gavage in rats or bolus dosing (capsules) in dogs, since these signs were not seen in the dietary studies. Therefore, the Meeting considered that these effects were not relevant for the establishment of an ARfD.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	104-week study of toxicity and carcinogenicity[a]	Toxicity	300 ppm, equal to 37.5 mg/kg bw per day	2000 ppm, equal to 272.4 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 272.4 mg/kg bw per day[c]	—

Species	Study	Effect	NOAEL	LOAEL
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	300 ppm, equal to 18.2 mg/kg bw per day	750 ppm, equal to 34 mg/kg bw per day[c]
		Carcinogenicity	750 ppm, equal to 34 mg/kg bw per day[c]	—
	Multigeneration study of reproductive toxicity[a]	Parental toxicity	300 ppm, equal to 32.3 mg/kg bw per day	1500 ppm, equal to 165.4 mg/kg bw per day[c]
		Offspring toxicity	300 ppm equal to 32.3 mg/kg bw per day	1500 ppm, equal to 165.4 mg/kg bw per day[c]
	Developmental toxicity[b]	Maternal toxicity	100 mg/kg bw per day[c]	—
		Embryo and fetal toxicity	100 mg/kg bw per day[c]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity	150 mg/kg bw per day	500 mg/kg bw per day[c]
		Embryo and fetal toxicity	500 mg/kg bw per day[c]	—

[a] Dietary administration.

[b] Gavage administration.

[c] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.2 mg/kg bw per day

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 azoxystrobin

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid and nearly complete absorption
Distribution	Widely distributed in tissues
Potential for accumulation	Low, no evidence of significant accumulation
Rate and extent of excretion	Approximately 82–90% (73–89% in faeces and 9–18% in urine) within 48 h
Metabolism in animals	Extensive; metabolic pathways include hydrolysis followed by glucuronide conjugation and minor pathway included cleavage of the ether
Toxicologically significant compounds (animals, plants and environment)	Azoxystrobin

Acute toxicity

Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	0.7 mg/l , dust (4 h exposure, nose only)
Rabbit, dermal irritation	Slight irritation
Rabbit, ocular irritation	Slight irritation
Guinea-pig, dermal sensitization	Not a sensitizer (Magnusson & Kligman test)

Short-term studies of toxicity

Target/critical effect	Body-weight effects
Lowest relevant oral NOAEL	20.4 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day; highest dose tested
Lowest relevant inhalation NOAEL	No data

Genotoxicity

	Unlikely to be genotoxic

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Liver-weight increases and bile-duct lesions
Lowest relevant NOAEL	18.2 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic in mice and rats

Reproductive toxicity

Reproduction target/critical effect	No toxicologically relevant effects
Lowest relevant reproductive NOAEL	165.4 mg/kg bw per day (rats; highest dose tested)
Developmental target/critical effect	No developmental toxicity in rats and rabbits
Lowest relevant developmental NOAEL	100 mg/kg bw per day (rats; highest dose tested)

Neurotoxicity/delayed neurotoxicity

Acute neurotoxicity	No sign of specific neurotoxicity

Mechanistic data

	No studies were submitted

Medical data

	No significant adverse health effects reported

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.2 mg/kg bw per day	Rat, 2-year study of toxicity	100
ARfD	Unnecessary	—	—

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BUPROFEZIN

First draft prepared by
Jürg Zarn[1] & Maria Tasheva[2]

[1]Nutritional and Toxicological Risks Section, Swiss Federal Office of Public Health, Zurich, Switzerland; and
[2]National Service for Plant Protection, Ministry of Agriculture and Food, Sofia, Bulgaria

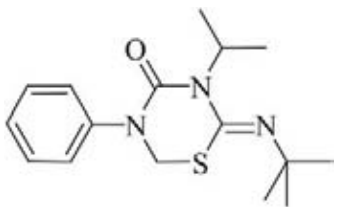
Explanation

Buprofezin is the ISO approved name for (*EZ*)-2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one (IUPAC), CAS No. 69327-76-0. Buprofezin is an insecticide that acts by the inhibition of chitin synthesis.

Buprofezin was previously evaluated by the JMPR in 1991 when an acceptable daily intake (ADI) of 0–0.01 mg/kg bw per day was established based on a no-observed-adverse-effect level (NOAEL) of 0.9 mg/kg bw per day identified in a 2-year study in rats and with a safety factor of 100. The JMPR in 1999 considered that the establishment of an acute reference dose (ARfD) was unnecessary. Buprofezin was re-evaluated by the present Meeting as part of the Codex Committee on Pesticide Residues (CCPR) periodic re-evaluation programme. New studies not previously evaluated by the Meeting included several studies of acute oral toxicity, irritation, sensitization and genotoxicity, metabolism studies in rats and a two-generation study in rats.

The more recent studies complied with good laboratory practice (GLP), but many of the older reported studies were performed before the widespread use of GLP.

Figure 1. Chemical structure of buprofezin



Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

The absorption, metabolism, distribution and excretion of [phenyl-^{14}C]buprofezin (radiochemical purity, > 97%; specific activity, 0.074 or 0.833 GBq/mmol) were investigated in fasted male Sprague-Dawley rats given single oral doses of buprofezin at 10 or 100 mg/kg bw. Additionally, bile-duct cannulated rats were given buprofezin at a dose of 10 mg/kg bw. Neither a GLP nor a quality assurance statement was included.

Irrespective of the dose administered, excretion was complete within 2–3 days after administration (Table 1), with 22–25% found in the urine and 70–74% in the faeces. In the bile-duct cannulated rats, 33.2% of the administered dose was excreted in the bile.

Absorption peaked at 9 h after administration and excretion was biphasic with half-lives of 13 h and 60 h (Table 3). C_{max} was approximately proportional to the dose administered, suggesting that absorption was not saturated in this dose range. Within 2 h, buprofezin was widely distributed into the organs, with the highest concentrations of radiolabel measured in adipose tissue, kidney, liver and urinary bladder at most of the time-points between 2 h and 96 h (Table 4). At 10 mg/kg bw, in the liver only, more than 1 µg equivalents/g were found at 96 h. In the group at the lower dose, elimination from the thyroid was slower than from other organs. At the higher dose, concentrations of radiolabel in many organs were higher than proportional to the administered dose at several time-points but not at study termination. There was no evidence for accumulation of radiolabel in any organ. The results of the organ analysis were essentially confirmed by whole-body autoradiography performed at the same time-points as organ analysis.

The metabolism of buprofezin was studied in rat liver homogenates and in urine, faeces and bile in vivo. Hydroxylation with consecutive methylation of the phenyl ring, oxidation of sulfur with consecutive ring opening of the thiadiazinane ring and conjugation reactions with sulfate and

glucuronic acid were the main metabolic routes. Besides the conjugates, the metabolites buprofezin (BF1), buprofezin sulfoxide (BF10), isopropylphenylurea (BF12), 4-hydroxybuprofezin (BF2), dihydroxy-buprofezin (C), hydroxy-methoxy-buprofezin (BF27) and 2-hydroxyisopropylphenylurea (BF13) (only in vitro) were identified (Figure 2) (Sugimoto, 1982).

In a supplementary study, previously not-analysed polar metabolites detected in male rats given buprofezin at a dose of 10 or 100 mg/kg bw (Sugimoto, 1982) were further characterized. This lead to the identification of these metabolites as glucuronic acid and/or sulfuric acid conjugates of 4-aminophenol (BF22, K) (0.2% in urine at 10 mg/kg bw) and 4-hydroxyacetanilide (BF23) (4.1% of administered dose in the urine and 2.7% of the administered dose in the faeces at 10 mg/kg bw) (Figure 2) (Uchida, 1988).

Table 1. Cumulative excretion of radiolabel in male rats given a single oral dose of radiolabelled buprofezin

Dose (mg/kg bw)	Time after administration (days)	Recovery (% of administered dose)			
		Urine	Faeces	Expired	Total
10	0.5	11.87	24.11	0.05	36.03
	1	18.91	63.23	0.16	82.3
	2	21.28	72.46	0.19	93.93
	3	21.69	73.58	0.20	95.47
	4	21.91	73.95	0.21	96.07
100	0.5	12.10	15.14	0.07	27.31
	1	18.81	41.75	0.15	60.71
	2	24.00	67.01	0.19	91.20
	3	24.68	69.95	0.20	94.83
	4	25.20	70.45	0.21	95.86

From Sugimoto (1982)

Table 2. Recovery of radiolabel in the blood of male rats given a single oral dose of radiolabelled buprofezin

Time after administration (h)	Concentration of radiolabel (µg equivalents/g)	
	Dose (mg/kg bw)	
	10	100
0.5	0.122	0.976
1	0.169	1.476
2	0.268	2.594
4	0.485	6.154
6	0.941	9.442
9	1.164	13.818
12	1.080	12.553
15	0.933	10.142
24	0.576	5.930
48	0.375	4.084
96	0.245	2.472

From Sugimoto (1982)

Table 3. Blood pharmacokinetic parameters in male rats given a single oral dose of radiolabelled buprofezin

Dose (mg/kg bw)	t_{max} (h)	c_{max} (µg/ml)	$t_{1/2\ e(9–24h)}$ (h)	$t_{1/2\ e(24–96)}$ (h)
10	9	1.164	13	60
100	9	13.282	13	60

From Sugimoto (1982)

Table 4. Recovery of radiolabel from organs of male rats given a single oral dose of radiolabelled buprofezin

Organ/tissue	Dose(mg/kg bw)										Ratio of recovery, 100 mg/kg bw: 10 mg/kg bw				
	Concentration of radiolabel (µg equivalents/g or ml)														
	10					100									
	Time after administration (h)					Time after administration (h)					Time after administration (h)				
	2	5	9	24	96	2	5	9	24	96	2	5	9	24	96
Adipose	0.8	4.0	4.2	1.7	0.1	23.6	86.8	114.7	46.0	1.6	31	21	28	27	14
Adrenal	0.4	2.3	1.6	0.5	0.1	10.6	43.5	47.0	7.1	1.0	26	19	30	14	13
Blood	0.1	0.8	1.0	0.5	0.2	2.6	9.4	13.7	6.0	2.5	21	12	13	12	10
Bone	0.1	0.4	0.7	0.1	0.0	2.4	6.8	6.2	1.7	0.3	17	16	9	14	8
Brain	0.3	0.5	0.3	0.1	0.0	5.5	12.5	10.4	1.2	0.3	21	25	37	16	12
Eye ball	0.2	0.2	0.2	0.1	0.0	1.5	5.2	5.3	1.0	0.2	9	27	23	13	11
Heart	0.3	0.7	0.6	0.2	0.1	6.9	12.9	14.7	2.4	0.5	21	18	23	11	10
Intestine	NA	NA	NA	NA	0.3	NA	NA	NA	NA	3.8	NA	NA	NA	NA	11
Kidney	1.2	2.3	2.5	0.7	0.3	16.7	35.8	33.6	8.1	2.1	14	16	13	12	8
Liver	4.9	11.2	8.4	3.2	1.2	44.3	70.3	85.5	25.9	8.0	9	6	10	8	7
Lung	0.4	0.8	0.7	0.4	0.1	5.7	18.6	14.8	3.8	0.9	16	23	20	11	9
Muscle	0.2	0.3	0.4	0.1	0.0	3.7	9.6	9.1	1.5	0.4	21	28	24	17	16
Pancreas	0.6	1.2	1.3	0.3	0.0	15.1	32.8	31.4	6.4	0.4	24	28	25	21	16
Salivary gland	0.4	0.6	0.6	0.2	0.0	5.1	14.8	14.8	2.8	0.4	14	24	24	15	10
Spleen	0.3	0.6	0.6	0.3	0.1	6.9	32.8	13.3	3.0	0.6	23	51	22	12	12
Testis	0.2	0.4	0.3	0.1	0.0	3.7	8.6	8.2	1.0	0.3	23	21	25	11	11
Thymus	0.2	0.5	0.4	0.2	0.0	4.0	10.9	11.3	1.7	0.3	24	23	27	11	17
Thyroid	0.4	0.9	0.7	0.6	0.5	10.6	22.1	17.6	5.5	0.7	26	24	24	9	1
Urinary bladder	5.3	9.7	14.3	2.1	0.1	53.8	49.2	70.7	15.7	1.6	10	5	5	8	11

From Sugimoto (1982)

NA, not analysed.

The absorption, distribution, metabolism and excretion of [phenyl-^{14}C]buprofezin (radiochemical purity, > 97%; specific activity, 0.798 GBq/mmol) was investigated in male and female CD rats. Groups of five male and five female rats were given a single dose of radiolabelled buprofezin at 10 mg/kg bw or 100 mg/kg bw by oral gavage. Urine and faeces were collected at 6 h, 24 h and thereafter every 24 h until terminal sacrifice at 168 h. After sacrifice, organs were retained for radioactivity analysis. An additional group of three male and four female rats were bile-duct cannulated and given a single dose of radiolabelled buprofezin at 10 mg/kg bw. Urine, faeces and bile were collected for

24 h. A statement of GLP compliance was not included in the study report but a statement of quality assurance was provided.

At both doses, most of the radioactivity was excreted in the faeces within 48 h; urinary excretion accounted for 20–22% in males and 13–15% in females (Table 5). Excretion initially was slightly faster in males than in females and was completed after 168 h in males and females.

At both doses in males and females, the highest concentrations of radiolabel were found in blood cells, the thyroid and the liver (Table 6). Maximal concentrations in these organs were 0.36 µg equivalents/g at the lower dose and 2.3 µg equivalents/g at the higher dose. Less than 0.7% of the administered dose was recovered in the body at terminal sacrifice of rats at either dose.

In the bile-duct cannulated rats, only 2.6–5.5% of the administered dose was found in the urine (Table 7); this is about one quarter of the amount found in non-cannulated rats. In the bile, approximately 30–40% of the administered dose was excreted within 24 h. Therefore, there was evidence for enterohepatic recirculation of buprofezin. Since the concentration of radiolabel in the gastrointestinal tract of females was higher than in males, absorption in females might be slower than in males.

Thin-layer chromatography (TLC) of samples of urine and bile indicated a high degree of conjugation, while acid hydrolysis of faecal extracts revealed unchanged buprofezin (BF1), 4-hydroxy-buprofezin (BF2) and possibly dimethoxy-buprofezin (BF20, A-15) (Caley & Cameron, 1988).

Table 5. Recovery of radiolabel in rats given a single dose of radiolabelled buprofezin by oral gavage

Time interval	Concentration of radiolabel (mean % of administered dose)											
	Dose (mg/kg bw)											
	10						100					
	Urine		Faeces		Total		Urine		Faeces		Total	
	M	F	M	F	M	F	M	F	M	F	M	F
0–24 h	18.5	10.8	60.7	53.5	79.2	64.4	18.1	11.8	57.4	48.5	75.5	60.3
0–48 h	20.1	12.6	71.0	76.0	91.1	88.5	20.6	13.7	69.3	73.0	90.0	86.7
0–168 h	20.9	13.4	72.8	79.1	94.8	93.4	21.7	14.6	72.7	85.1	95.2	100.2

From Caley & Cameron (1988)

F, females; M, males

Table 7. Distribution of radiolabel in bile-duct cannulated rats given a single dose of radiolabelled buprofezin at 10 mg/kg bw by oral gavage

Tissue/body fluid	Recovery of radiolabel (% of administered dose)	
	Males	Females
Bile	29.8	38.2
Urine	5.5	2.6
Faeces	34.0	19.0
Gastrointestinal tract	16.6	27.5
Liver	1.0	1.5
Carcass	3.8	3.2
Total	90.9	94.5

From Caley & Cameron (1988)

Table 6. Recovery of radiolabel at 168 h from organs of rats given a single dose of radiolabelled buprofezin by oral gavage

Organ	Dose (mg/kg bw)							
	10				100			
	Males		Females		Males		Female	
	µg equivalents/g	% of dose	µg equivalents/g	% of dose	µg equivalents/g	% of dose	µg equivalents/g	% of dose
Bone	0.023	—	0.017	—	0.207	—	0.128	—
Fat	0.031	—	0.028	—	0.316	—	0.252	—
Skeletal muscle	0.024	—	0.017	—	0.229	—	0.205	—
Brain	0.014	0.001	0.016	0.001	0.228	0.002	0.190	0.001
Heart	0.032	0.002	0.030	0.001	0.531	0.002	0.443	0.001
Liver	0.313	0.182	0.360	0.151	2.198	0.128	2.053	0.091
Lung	0.048	0.004	0.054	0.003	0.528	0.003	0.547	0.005
Spleen	0.034	0.001	0.034	0.001	0.441	0.001	0.328	0.001
Kidney	0.068	0.007	0.074	0.007	0.618	0.007	0.532	0.004
Testes/ovaries	0.014	0.002	0.042	0.000	0.095	0.001	0.304	0.000
Uterus	—	—	0.032	0.001	—	—	0.283	0.001
Thyroid	0.155	0.000	0.359	0.000	1.831	0.000	2.342	0.001
Stomach + contents	0.010	0.003	0.013	0.002	0.071	0.003	0.070	0.001
Intestine + contents	0.027	0.033	0.036	0.034	0.230	0.029	0.195	0.018
Plasma	0.026	—	0.031	—	0.365	—	0.377	—
Blood cells	0.150	—	0.140	—	2.278	—	1.855	—

From Caley & Cameron (1988)

— Not calculable, according to the study report

In an accumulation study, two groups of 27 young male rats were given diets containing technical buprofezin (purity, 99%) at a concentration of 200 or 1000 ppm. At 2 and 4, days and 1, 2, 4, 8, 12, 16 and 24 weeks, three rats were sacrificed and buprofezin content of the organs was analysed. The limit of detection (LOD) in the organs was 0.1 ppm. No statements of compliance with GLP or quality assurance were provided.

At 200 ppm, buprofezin was found occasionally at very low concentrations that were slightly above the LOD. In adipose tissue from day 4 onwards, concentrations of buprofezin were up to approximately 1 ppm. In the groups at 1000 ppm, concentrations of buprofezin in the liver were up to approximately 1 ppm and in adipose tissue up to approximately 10 ppm. There was no evidence for accumulation of buprofezin, but since only buprofezin was analysed, no conclusion about the metabolite profiles in the tissues can be drawn (Sugaya & Uchida, 1980).

To study the metabolism of buprofezin in rats, five males were given a single oral dose of [phenyl-^{14}C]buprofezin (radiochemical purity, 99%; specific activity, 0.601 GBq/mmol) at 100 mg/kg bw. Urine and faeces were collected until terminal sacrifice at 72 h after dosing; metabolites in organs were analysed at study termination. The study complied with GLP.

Renal excretion was virtually complete within 48 h and accounted for 12.9% and faecal excretion for 79.1% of the administered dose; less than 0.8% remained in the body. The highest concentrations of residual radioactivity were found in kidney (1 µg equivalents/g), blood, thyroid and liver (7 µg equivalents/g).

Of the administered dose, 60.7% could be allocated to individual metabolites and no unidentified individual metabolite exceeded 3.7% (Table 8). The metabolic pathways include phenyl-ring hydroxylation, oxidation of the t-butyl group and thiadiazinane ring-opening with intensive conjugation of metabolites. Buprofezin and BF27 were identified after extraction of faeces and BF9, BF10, BF12, BF13 after hydrolysis of unextractable faecal metabolites. BF13 and BF23, BF28 conjugates were identified as urine residues after sulfatase cleavage. Figure 2 depicts the metabolic scheme in the rat (Huang & Smith, 1997).

In a supplemental metabolism study designed to identify early metabolites in the organs, a single oral dose of 100 mg/kg bw [phenyl-^{14}C]buprofezin (radiochemical purity, not specified; specific activity, 1.13 GBq/mmol) was given to 12 male Sprague Dawley rats. Groups of four rats were killed at 3, 6 and 72 h and metabolites in the liver, kidney, heart and plasma and the content of the gastrointestinal tract were identified. Faeces and urine were collected throughout the study and analysed for metabolites. To investigate immediate early metabolism, rat liver microsomes were incubated with 2 µmol/l of [phenyl-^{14}C]buprofezin and after 10 and 30 min, respectively, the metabolites were extracted and investigated. The study complied with GLP.

Apart from a range of previously identified rat metabolites, the liver homogenates were found to contain the metabolites tert-butylhydroxy-buprofezin (BF4), phenylbiuret (BF11) and thiobiuret (BF25) (Table 9). In the in-vivo study, newly identified metabolites included BF4 in liver kidney, heart and the gastrointestinal tract, BF11 was found in liver and kidney and BF25 in the liver only (Yoshizane, 2008).

Conjugates are not shown; structures in brackets were not found in rats but are proposed intermediates; BF26 was found in plants.

1.2 Effects on enzymes and other biochemical parameters

No information was available.

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with buprofezin are summarized in Table 1.

Table 8. Identification of metabolites in rats given a single oral dose of radiolabelled buprofezin

Metabolite	Recovery (% of administered dose)	
	Urine	Faeces
Buprofezin (BF1)	ND	45.4
Dione metabolite (BF9)	ND	< 0.1
Buprofezin sulfoxide (BF10)	ND	0.1
Isopropylphenylurea (BF12)	ND	0.1
4-Hydroxyisopropylphenylurea (BF13)	0.5	< 0.1
4-Hydroxyacetanilide (BF23)	2.5	ND
Hydroxy-methoxy-buprofezin (BF27)	ND	7.2
2-[3-Isopropyl-3-[methylsulfonylmethyl(phenyl)carbamoyl]ureido]-2-methylpropionic acid (BF28)	0.3	4.6

From Huang & Smith (1997)

ND, not detected

Table 9. Recovery of metabolites found in male rat liver microsomes in vitro[a] and in organs[b] of rats given a single oral dose of radiolabelled buprofezin

Metabolite	Recovery (% of total radioactive residue)				
	Microsomes	Liver	Kidney	Heart	Gastrointestinal tract
Buprofezin (BF1)	39.22	12.2	17.03	30.25	47.34
4-Hydroxybuprofezin (BF2)	28.22	8.02	8.25	14.6	0.09
Tert-butylhydroxy-buprofezin (BF4)	10.99	7.97	1.42	2.06	0.68
Dione metabolite (BF9)	0.04	0.11	ND	ND	ND
Phenylbiuret (BF11)	0.04	0.27	0.14	ND	ND
Isopropylphenylurea (BF12)	ND	0.56	ND	ND	0.03
Thiobiuret (BF25)	0.17	0.09	ND	ND	ND
2-Amino-2-methylpropyl-2-methylethy *l*-4-Phenyl-allophanate (BF26)	ND	ND.	ND	ND	ND
Hydroxy-methoxy-buprofezin (BF27)	ND	2.60	10.69	25.82	1.42
Others	8.05	8.82	13.76	14.2	2.34

From Yoshizane (2008)

ND, not detected

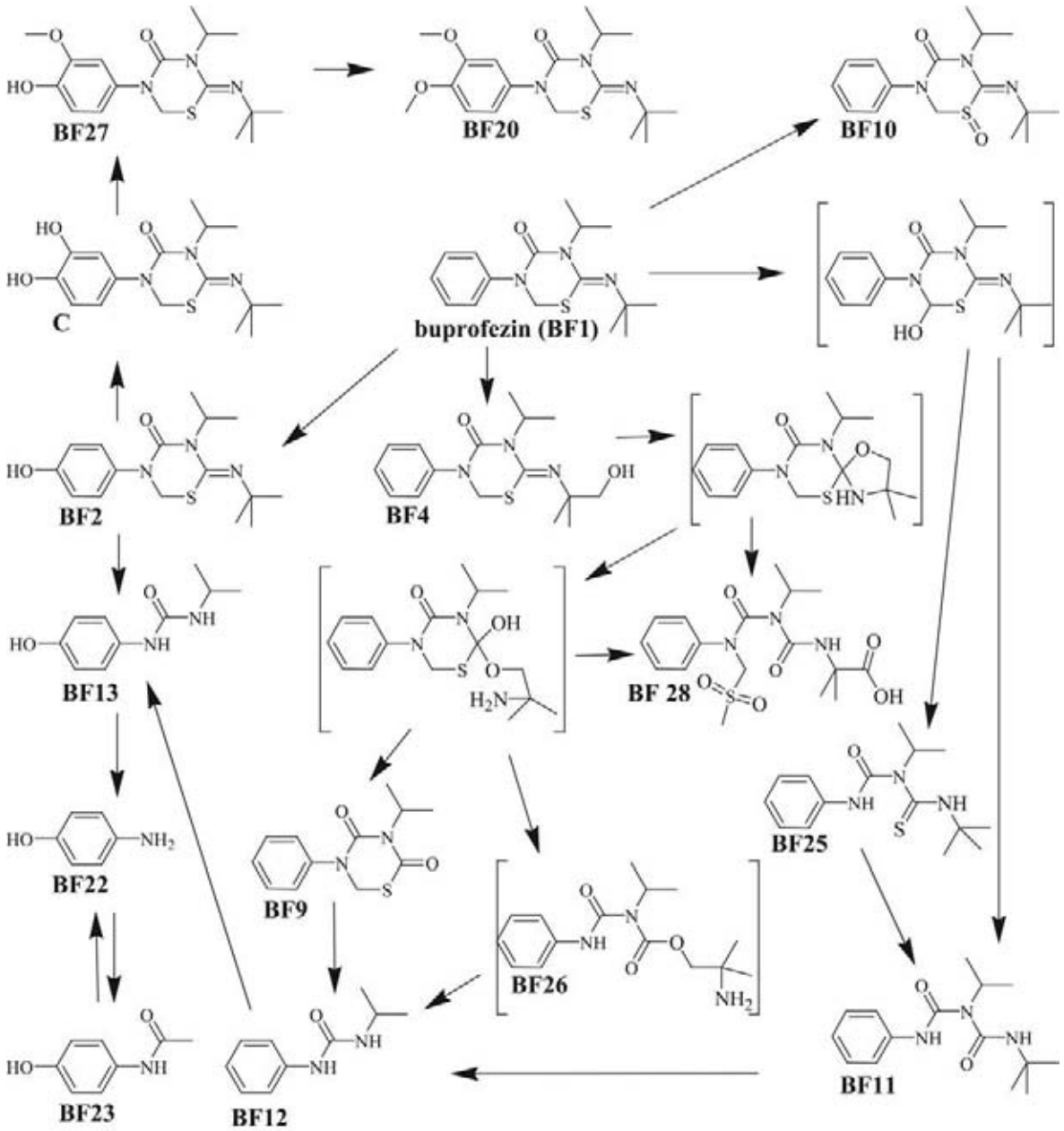
[a] After incubation with [phenyl-^{14}C]buprofezin for 10 min

[b] Rats killed and samples taken 3 h after a single oral dose of [phenyl-^{14}C]buprofezin at 100 mg/kg bw

Table 10. All identified rat metabolites of buprofezin

Metabolite		References
BF1	buprofezin	Sugimoto (1982); Caley & Cameron (1988); Huang & Smith (1997); Yoshizane (2008)
BF2	4-hydroxybuprofezin	Sugimoto (1982); Caley & Cameron (1988); Yoshizane (2008)
BF4	tert-butylhydroxy-buprofezin	Yoshizane (2008)
BF9	dione metabolite	Huang & Smith (1997); Yoshizane (2008)
BF10	buprofezin sulfoxide	Sugimoto (1982); Huang & Smith (1997)
BF11	phenylbiuret	Yoshizane (2008)
BF12	isopropylphenylurea	Sugimoto (1982); Huang & Smith (1997); Yoshizane (2008)
BF13	4-hydroxyisopropylphenylurea	Sugimoto (1982); Huang & Smith (1997)
BF20	dimethoxy buprofezin	Caley & Cameron (1988)
BF22	4-aminophenol	Uchida (1988)
BF23	4-hydroxyacetanilide	Uchida (1988); Huang & Smith (1997)
BF25	thiobiuret	Yoshizane (2008)
BF27	hydroxy-methoxy-buprofezin	Sugimoto (1982); Huang & Smith (1997); Yoshizane (2008)
BF28	2-[3-isopropyl-3-[methylsulfonylmethyl(phenyl) carbamoyl] ureido]-2-methylpropionic acid	Huang & Smith (1997)
C	dihydroxy buprofezin	Sugimoto (1982)

Figure 2. Proposed metabolic pathway of buprofezin in rats



(a) Oral administration

Groups of 10 male and 10 female ICR mice were given buprofezin (purity, > 99.5%) as a single oral dose at 2500, 5000 or 10000 mg/kg bw by gavage.

No mice died at any dose. Female mice at 2500 mg/kg bw and above and males at 10 000 mg/kg bw showed reduced locomotor activity within the first 2 days after dosing, and males also had loose stool (Ebino & Shirasu, 1981a).

Groups of 10 male Golden hamsters were given buprofezin (purity, > 99%) as a single dose at 7692 or 10000 mg/kg bw by oral gavage.

No hamsters died at either dose and no clinical signs of toxicity were observed (Tsuchiya & Sugimoto, 1979a).

Groups of 10 male and 10 female Fischer rats were given buprofezin (purity, > 99.5%) as a single dose at 1412, 1765, 2206, 2758 or 3447 mg/kg bw by oral gavage. In all treated groups, decreased locomotor movement and lacrymation were observed. At doses of ≥ 1765 mg/kg bw, duodenum ulceration and duodenum oedema were observed. At ≥ 1765 mg/kg bw, mortality was observed and the median lethal dose (LD_{50}) was calculated to be 2198 mg/kg bw in males and 2355 mg/kg bw in females (Ebino & Shirasu, 1981b).

Groups of five male and five female Sprague-Dawley rats were given buprofezin (purity, 99.6%) as a single dose at 1000, 1400, 1960, 2744 or 3842 mg/kg bw by oral gavage in the first of a two-part experiment. The study was performed according to GLP requirements.

Clinical signs in all treated groups consisted of decreased locomotor activity, orbital and nasal discharge within 3 to 6 h after dosing. Tremor, lacrymation and loose stool were also seen between 6 h and 1 day. One male at the highest dose died.

In the second part of the experiment, groups of five male and five female Sprague-Dawley rats were given a single dose of buprofezin at 2959, 3846, 5000, 6500 or 8450 mg/kg bw. The LD_{50} in this experiment was 3847 mg/kg bw in males and 2278 mg/kg bw in females (Komatsu, 1996).

Groups of 10 male and 10 female Sprague-Dawley rats were given buprofezin (purity, > 99%) as a single dose at 1021, 1429, 2000, 2800, 3920 or 5488 (males only) mg/kg bw by oral gavage.

Clinical signs in treated rats included decreased locomotor activity, lacrymation, urine incontinentia and diarrhoea and at necropsy duodenal ulcers were also found. The LD_{50} was 1635 mg/kg bw in males and 2015 mg/kg bw in females (Tsuchiya & Sugimoto, 1982).

Two male Japanese albino rabbits were given buprofezin (purity, > 99%) as a single dose at 5000 mg/kg bw by oral gavage.

No clinical signs of toxicity and no mortality were observed (Tsuchiya & Sugimoto, 1979b).

(b) Intraperitoneal administration

Groups of 10 male and 10 female ICR mice were given buprofezin (purity, > 99.5%) as a single intraperitoneal dose at 2500, 5000 or 10 000 mg/kg bw.

No mice died at any dose. Males at 5000 mg/kg bw and above and females at 10 000 mg/kg bw had swollen livers (Ebino & Shirasu, 1981a).

Groups of 10 male and 10 female Fischer rats were given buprofezin (purity, > 99.5%) as a single intraperitoneal dose of 2500, 5000 or 10 000 mg/kg bw.

No mortalities were observed. At all doses, male and female mice had swollen livers and kidneys and males also had lung petechia (small haemorrhagic spots) (Ebino & Shirasu, 1981b).

(c) Dermal application

Groups of 10 male and 10 female Fischer rats were given buprofezin (purity, > 99.5%) as a single dermal application at 1000, 2000 or 5000 mg/kg bw.

No clinical signs of toxicity or mortalities were observed (Ebino & Shirasu, 1981b).

(d) Subcutaneous application

Groups of 10 male and 10 female ICR mice were given buprofezin (purity, > 99.5%) as a single subcutaneous dose at 2500, 5000 or 10 000 mg/kg bw.

No mice died at any dose. Apart from an intermittent decrease in body weight in two males and eight females at 10 000 mg/kg bw, no treatment-related findings were recorded (Ebino & Shirasu, 1981a).

Groups of 10 male and 10 female Fischer rats were given buprofezin (purity, > 99.5%) as a single subcutaneous dose at 2500, 5000 or 10 000 mg/kg bw.

No mortalities or clinical signs of toxicity were observed (Ebino & Shirasu, 1981b).

(e) Exposure by inhalation

Groups of 10 male and 10 female Fischer rats were exposed for 4 h to buprofezin (purity, 99.6%) at a dose of 3.57 or 4.57 mg/l.

All rats showed blood-like red staining around the nose. One female rat in the group at the higher dose was found dead on day 2 (Tsuda, 1984).

(f) Dermal and ocular irritation

Six male New Zealand White rabbits were exposed dermally to 0.5 g of buprofezin (purity, 99.3%) in 0.5 ml of 0.9% saline for 4 h. The study was conducted according Japanese GLP requirements.

No clinical signs of toxicity and no mortalities were observed (Blaszcak, 1986b).

Six male New Zealand White rabbits were given 42.5 mg of ground buprofezin (purity, 99.3%) in the right eye for 24 h; the eye was then washed. The study was conducted according Japanese GLP requirements.

Within the first hour after application, signs of conjunctival irritation and iridial changes were observed. In five rabbits, clinical signs had resolved within 24 h and in one rabbit within 72 h (Blaszcak, 1986a).

(g) Dermal sensitization

In a maximization test in female Hartley Albino guinea-pigs, buprofezin showed an equivocal potential for skin sensitization. In the challenge phase of the study, very minor initial signs of a dermal response, which was greater than that shown by the concurrent controls but which did not reach score 1 of the applied scoring system, were recorded in 50% of the guinea-pigs. In the re-challenge phase, 30% of the guinea-pigs also showed very minor signs of dermal reaction (Blaszcak, 1987).

Buprofezin (purity, 99.9%) was tested in a local lymph node assay (LLNA) that complied with GLP requirements. Groups of five female CBA/JNCrj mice were given 25 µl of buprofezin at a concentration of 6.25%, 12.5% or 25.0% topically for three consecutive days.

Since stimulation was very low at all concentrations and did not follow a dose–response relationship the Meeting concluded that buprofezin gave negative results in the LLNA (Inagaki, 2006b).

2.2 Short-term studies of toxicity

Rats

Groups of five male and five female CD rats were exposed to buprofezin (purity, 99%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day by dermal application for at least 6 h per day for 24 days. An additional five males and five females in the control group and at the highest dose served as reversibility groups. Rats were observed twice per day for clinical signs of toxicity, and body weights and feed consumption were recorded regularly. Haematology, clinical chemistry and urine analysis investigations were performed towards the end of the study. At terminal sacrifice, macroscopic examination of the organs was performed and selected organs were weighed and prepared for histology. The study complied with GLP.

In females at the highest dose, absolute and relative kidney weights were increased at study termination in the main study group but not in the reversibility group. In three males at the highest dose, focal necrosis with inflammatory infiltrate in the liver was observed; in all other groups of males or females, the incidence of this finding was one or zero. In males at the highest dose, there was more hyperkeratosis in the skin than in males in the control group.

Table 11. Acute toxicity of buprofezin

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%)	Reference
Mouse	ICR	M/F	Oral	> 10 000	—	> 99.5	Ebino & Shirasu (1981a)
Mouse	ICR	M/F	Intraperitoneal	> 10 000	—	> 99.5	Ebino & Shirasu (1981a)
Mouse	ICR	M/F	Subcutaneous	> 10 000	—	> 99.5	Ebino & Shirasu (1981a)
Hamster	Golden-	M	Oral	> 10 000	—	> 99.0	Tsuchiya & Sugimoto (1979a)
Rat	Fischer	M	Oral	2 198	—	> 99.5	Ebino & Shirasu (1981b)
Rat	Fischer	F	Oral	2 355	—	> 99.5	Ebino & Shirasu (1981b)
Rat	Fischer	M/F	Subcutaneous	> 10 000	—	> 99.5	Ebino & Shirasu (1981b)
Rat	Fischer	M/F	Intraperitoneal	> 10 000	—	> 99.5	Ebino & Shirasu (1981b)
Rat	Fischer	M/F	Dermal	> 10 000	—	> 99.5	Ebino & Shirasu (1981b)
Rat	SD	M	Oral	3 847	—	99.6	Komatsu (1996)
Rat	SD	F	Oral	2 278	—	99.6	Komatsu (1996)
Rat	SD	M	Oral	1 635	—	> 99.0	Tsuchiya & Sugimoto (1982)
Rat	SD	F	Oral	2 015	—	> 99.0	Tsuchiya & Sugimoto (1982)
Rabbit	Japanese albino	M	Oral	> 5 000	—	> 99.0	Tsuchiya & Sugimoto (1979b)
Rat	Fischer	M/F	Inhalation	—	> 4.57	99.6	Tsuda (1984)

F, female; M, male; SD, Sprague-Dawley

Since the findings in this study were not clearly treatment-related and of questionable toxicological relevance, the NOAEL was 1000 mg/kg bw per day (Willoughby, 1995).

Groups of 10 male and 10 female Jcl:SD rats were fed diets containing buprofezin (purity, > 99%) at a concentration of 0, 40, 200, 1000 or 5000 ppm (equal to 0, 3.4, 13.0, 68.5 and 316.2 mg/kg bw per day in males and 0, 4.1, 16.3, 81.8 and 362.2 mg/kg bw per day in females, respectively) for 13 weeks. The rats were observed twice per day for clinical signs of toxicity, and body weights were recorded weekly and feed intake was noted twice per week. Ophthalmological examinations were done at week 12 and urine analysis, haematology and clinical chemistry parameters were investigated at study termination. Organs were weighed and macroscopically and histologically examined. This study complied with GLP.

There were no deaths and no treatment-related clinical signs in any group. In males, feed intake was statistically significantly reduced at 200 ppm and above, beginning from week 2 at 200 ppm and from week 1 at higher doses. In females, feed intake was lower at 5000 ppm. In males and females at 5000 ppm, body weights were statistically significantly lower than those of the controls from the first week of treatment. At study termination, body weights were lower by 9% and 14% in males and females, respectively. In the urine of male rats, increased numbers of round epithelial cells were found at 1000 and 5000 ppm. In males, concentrations of haemoglobin, hematocrit (erythrocyte volume fraction) and erythrocyte and monocyte counts were statistically significantly reduced at 5000 ppm and activated partial thromboplastin time was prolonged. For females, all except rats at the highest dose and rats in the control group had increased erythrocyte counts and in all rats treated with buprofezin MCV and MCH were lowered. In females at the highest dose, activated partial thromboplastin time was prolonged and neutrophil counts were reduced at dietary concentrations of 200 ppm and above.

In males at 200 ppm and above and in females at 5000 ppm, glucose concentrations were statistically significantly reduced. Triglyceride concentrations were lowered at 5000 ppm in males and females, and cholesterol, phospholipids and blood urea nitrogen increased. In females at the highest dose, cholinesterase activity was slightly reduced. In females at 1000 ppm and above, α-and β-globulins were increased, as was albumin at 5000 ppm. Albumin and α- and β-globulins were increased in males at 5000 ppm and γ-globulins were lowered.

At 5000 ppm in males and females, thyroids and livers were enlarged and livers had a dark brownish appearance. In males at 5000 ppm, caeca were enlarged and kidneys appeared dark brownish in colour. Treatment-related organ-weight changes included increases in absolute and relative liver and thyroid weights and decreases in absolute and relative spleen weights in males and females at 5000 ppm (Table 12). Liver weights in females were also increased at 1000 ppm. In males and females at 5000 ppm, absolute heart and lung weights were statistically significantly decreased but not the relative weights, while relative brain weights were increased but not absolute brain weights.

In the livers of males and females at 5000 ppm, hepatocellular hypertrophy, enlarged hepatocellular nuclei and nucleoli, focal infiltration of small round cells and, additionally, in females single-cell and focal hepatocellular necrosis and focal infiltration of small round cells were observed (Table 13). The thyroids of males and females at dietary concentrations of 1000 ppm and above showed thickening and hyperplasia of follicular epithelial cells. In males at 200 ppm and above, fatty changes of the proximal tubular epithelium in the kidney were seen without a clear dose–response relationship.

The NOAEL was 40 ppm, equal to 3.4 mg/kg bw per day, on the basis of decreased feed intake in males and mild clinical chemistry changes at 200 ppm (Watanabe, 1986).

Table 12. Body weights, absolute organ weights and relative organ weights for rats given diets containing buprofezin for 13 weeks

Dietary concentration (ppm)	Body weight (g)	Organ weights					
		Liver		Spleen		Thyroid	
		Absolute (g)	Relative (%)	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
Males							
0	536	16.702	3.11	0.958	0.18	0.021	0.0039
40	533	15.139	2.83*	0.869	0.16	0.023	0.0042
200	519	14.597*	2.82*	0.896	0.17	0.022	0.0042
1000	517	16.490	3.19	0.866	0.17	0.025	0.0048*
5000	484**	21.995**	4.54**	0.738**	0.15*	0.043**	0.0088**
Females							
0	272	7.825	2.88	0.549	0.20	0.016	0.0060
40	269	7.377	2.75	0.520	0.19	0.016	0.0058
200	266	7.467	2.80	0.511	0.19	0.016	0.0060
1000	258	8.270	3.22*	0.479	0.19	0.017	0.0067
5000	238**	10.539**	4.44**	0.413**	0.17**	0.038**	0.0159**

From Watanabe (1986)

* $p < 0.05$

** $p < 0.01$

Table 13. Histological changes in rats given diets containing buprofezin for 13 weeks

Dietary concentration (ppm)	Incidence of histological changes									
	Males (n = 10)					Females (n = 10)				
	0	40	200	1000	5000	0	40	200	1000	5000
Liver										
Single-cell necrosis	1	1	5	0	0	1	1	2	0	5
Focal necrosis	0	1	0	0	1	0	0	0	0	5
Hepatocellular hypertrophy	0	0	0	2	10	0	0	0	0	10
Enlargement of hepatocellular nuclei	0	0	0	0	10	0	0	0	2	8
Enlargement of hepatocellular nucleoli	0	0	0	0	7	0	0	0	1	7
Focal infiltration of small round cells	0	0	0	0	0	1	0	1	0	5
Fat-loaded hepatocytes	0	1	0	0	5	0	1	2	3	2
Kidney										
Fatty change of proximal tubular epithelium	3	4	6	7	8	8	2	7	4	2
Thyroid										
Thickening/hyperplasia of follicular epithelial cells	0	0	0	10	10	0	0	0	9	10

From Watanabe (1986)

Dogs

Groups of four male and four female beagle dogs were given capsules containing buprofezin (purity, 99%) at a dose of 0, 2, 10, 50 or 300 mg/kg bw per day for 13 weeks. Clinical signs, feed consumption and body-weight development were recorded regularly, haematology, blood chemistry and urine analysis were noted periodically and ophthalmology was performed towards the end of the study. After terminal sacrifice, organ weights were recorded and organs were examined macroscopically and histologically. The study complied with GLP.

Subdued behaviour was observed in one male at 50 mg/kg bw per day and in virtually all dogs at 300 mg/kg bw per day 1 h after treatment and persisted throughout the day. This observation was made predominantly in the early part of the first week of treatment, but also at other time-points throughout the study. During the course of the study, the incidence of this finding clearly decreased. Subdued behaviour was also seen occasionally in two females at 2 mg/kg bw per day. In virtually all dogs at 300 mg/kg bw per day, slight ataxia (unsteadiness on feet) was observed 1 h after treatment and persisted for about 5 h. This observation was made early in the first week of treatment resolving in females in the first week but persisting with a lower incidence throughout the first nine weeks in one male. Two males and two females at the highest dose showed abdominal distension usually 1 h after treatment, which usually persisted for 4 h. This effect was also seen in one male in week 2 of treatment.

Dogs at the highest dose had slightly lower feed intake when compared to the pre-treatment period and to that of the controls and body-weight gains were statistically significantly decreased (Table 14). Prothrombin time in females at the highest dose was statistically significantly extended and the activity of alkaline phosphatase (ALP) was increased in males at 50 mg/kg bw per day and in males and females at the highest dose (Table 15). At the highest dose, alanine aminotransferase (ALT) activity was also slightly but statistically significantly increased. In all males receiving buprofezin, absolute and relative liver weights were increased. The effect was statistically significant for males and females at 50 and 300 mg/kg bw per day (Table 16). In males at doses of 50 mg/kg bw per day and above, absolute and relative thyroid weights were increased and in females the relative weights of the thyroid were increased at the highest dose. Absolute and relative kidney weights were increased in males at the highest dose; only the relative kidney weight was increased statistically significantly in females. On histological examination, homogeneity of hepatocellular cytoplasm was seen in all dogs at 300 mg/kg bw per day and in three males and three females at 50 mg/kg bw per day. Additionally, in all dogs at 300 mg/kg bw per day and in one female at 50 mg/kg bw per day, eosinophilic bodies in the hepatocellular cytoplasm were found.

The NOAEL in this study was 10 mg/kg bw per day, on the basis of increased ALP activity and liver- and thyroid-weight increases at 50 mg/kg bw per day (Broadmeadow, 1986).

Table 14. Mean terminal body-weight gain in dogs given capsules containing buprofezin for 13 weeks

Dose (mg/kg bw per day)	Body-weight gains ± standard deviation (kg)	
	Males	Females
0	4.3 ± 0.5	3.8 ± 0.6
2	3.8 ± 0.4	3.4 ± 0.5
10	3.7 ± 0.6	3.2 ± 0.5
50	3.5 ± 0.4	3.4 ± 0.3
300	3.2 ± 0.7*	2.0 ± 1.4**

From Broadmeadow (1986)

* $p < 0.05$

** $p < 0.01$

Table 15. Mean activity of alkaline phosphatase in dogs given capsules containing buprofezin for 13 weeks

Dose (mg/kg bw per day)	Alkaline phosphatase activity ± standard deviation (U/l)					
	Males			Females		
	Week –1	Week 6	Week 12	Week –1	Week 6	Week 12
0	105 ± 18	76 ± 17	68 ± 17	98 ± 18	72 ± 16	57 ± 12
2	121 ± 19	90 ± 12	82 ± 6	119 ± 25	85 ± 17	92 ± 39
10	110 ± 18	94 ± 17	94 ± 15	108 ± 19	85 ± 12	83 ± 11
50	103 ± 11	113 ± 20*	129 ± 30*	107 ± 22	96 ± 17	97 ± 21
300	105 ± 24	232 ± 43**	293 ± 62**	101 ± 12	218 ± 41**	281 ± 129**

From Broadmeadow (1986)

* $p < 0.05$

** $p < 0.001$

Table 16. Relative organ weights in in dogs given capsules containing buprofezin for 13 weeks

Dose (mg/kg bw per day)	Body weight (kg)	Relative organ weight ± standard deviation (% of body weight)		
		Liver	Kidney	Thyroid (× 1000)
Males				
0	14.4	3.1 ± 0.2	0.41 ± 0.02	5.4 ± 1.6
2	13.7	3.4 ± 0.3	0.41 ± 0.02	6.1 ± 0.6
10	13.7	3.8 ± 0.4	0.44 ± 0.02	6.9 ± 1.3
50	13.6	4.3 ± 0.6*	0.45 ± 0.03	9.0 ± 0.7**
300	12.9	5.2 ± 0.7**	0.51 ± 0.04**	11.3 ± 0.6**
Females				
0	13.3	3.1 ± 0.2	0.43 ± 0.04	6.7 ± 1.3
2	12.9	3.0 ± 0.2	0.42 ± 0.02	7.6 ± 1.7
10	12.8	3.3 ± 0.1	0.43 ± 0.03	7.2 ± 0.9
50	13.2	3.7 ± 0.2*	0.42 ± 0.04	7.6 ± 0.5
300	11.6	5.0 ± 0.5**	0.53 ± 0.03*	9.2 ± 1.4*

From Broadmeadow (1986)

* $p < 0.01$

** $p < 0.001$

Groups of six male and six female beagle dogs received oral capsules containing buprofezin (purity, ≥ 99.3%) at a dose of 0, 2, 20 or 200 mg/kg bw per day for 2 years (107 weeks). Clinical signs of toxicity, feed consumption, body-weight development, ophthalmology, haematology, blood chemistry parameters and urine analysis were recorded regularly. After terminal sacrifice, organ weights were recorded and organs were examined macroscopically and histologically. A statement of quality assurance was included.

No treatment-related clinical signs of toxicity were observed. Terminal body-weight gain was low in males and females at the highest dose but statistically significant only in females (Table 17). This effect in males was attributable to decreased body-weight gains in the first 6 months of administration and in females in the second year of the study. A trend in absolute liver-weight increases was seen in males at the intermediate dose and above and in all females receiving buprofezin, becoming statistically significant in females at the intermediate dose and above. Relative liver weights were increased in all treated females and in males at the highest dose (Table 17). Relative thyroid weights were increased in males and females at the highest dose. An elongated activated partial thromboplastin time in males at the highest dose only at week 26 and at study termination was considered to occur by chance. In males and females at the intermediate and the highest dose, ALP activity was statistically significantly increased when compared to that in dogs in the control group (Table 18). An electrophoretic isoenzyme analysis showed that hepatic ALP isoenzyme contributed most to the increased ALP activity. In the second year of treatment, the activity of ALT was also increased in dogs with high ALP activity. Unchanged bromosulfonphthalein retention time in all dogs receiving buprofezin indicated normal liver function. In males and females at 200 mg/kg bw per day, fluctuations in thyroxine concentrations with a trend to lower concentrations was observed. Many male and female dogs at the intermediate dose and the highest dose, but none at the lowest dose or in the control group, were found to have slight bile-duct hyperplasia. Slight hepatocellular hypertrophy was found in males at the intermediate dose and at the highest dose. Four and thee females at the intermediate and the highest dose, respectively, had mammary hyperplasia while this change was only seen in one dog in the control group and one dog at the lowest dose.

The NOAEL was at 2 mg/kg bw per day on the basis of liver-weight increases and correlating histological changes and clinical chemistry changes at 20 mg/kg bw per day (Cummins, 1982).

Table 17. Mean terminal body-weight gain and organ weights[a] in dogs given capsules containing buprofezin for 2 years

Dose (mg/kg bw per day)	Body-weight gain (kg)	Liver weight		Thyroid weight	
		Absolute (g)	Relative (%)	Absolute (g)	Relative (% × 1000)
Males					
0	3.8 ± 1.7	422 ± 62	3.4 ± 0.7	1.01 ± 0.38	8.0 ± 2.5
2	3.7 ± 0.9	416 ± 63	3.4 ± 0.4	1.10 ± 0.25	8.9 ± 1.7
20	3.4 ± 0.7	489 ± 71	3.9 ± 0.5	1.19 ± 0.14	9.6 ± 1.1
200	2.5 ± 1.1	484 ± 47	4.3 ± 0.4**	1.30 ± 0.32	11.4 ± 2.2**
Females					
0	4.5 ± 1.4	367 ± 42	2.8 ± 0.1	0.94 ± 0.21	7.2 ± 1.0
2	3.9 ± 1.0	414 ± 42	3.4 ± 0.4*	1.05 ± 0.33	8.6 ± 2.5
20	4.2 ± 0.9	458 ± 76*	3.7 ± 0.3***	1.26 ± 0.41	10.2 ± 2.5
200	2.5 ± 1.2**	477 ± 90**	4.4 ± 0.6***	1.35 ± 0.45	12.4 ± 3.4**

From Cummins (1982)

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

[a] ± standard deviation

Table 18. Mean activity of alkaline phosphatase in dogs given capsules containing buprofezin for 2 years

Dose (mg/kg bw per day)	Alkaline phosphatase activity (U/l ± standard deviation)			
	Week –1	Week 4	Week 52	Week 102
Males				
0	111 ± 14	94 ± 16	72 ± 33	45 ± 10
2	137 ± 39	119 ± 28	83 ± 22	57 ± 19
20	121 ± 4	125 ± 17*	134 ± 44*	108 ± 27**
200	110 ± 14	240 ± 121**	362 ± 239**	310 ± 372**
Females				
0	102 ± 26	78 ± 17	57 ± 10	56 ± 16
2	100 ± 7	83 ± 14	87 ± 25	68 ± 26
20	132 ± 38	159 ± 49**	200 ± 70**	166 ± 46**
200	112 ± 23	210 ± 66**	404 ± 147**	288 ± 52**

From Cummins (1982)

* $p < 0.05$

** $p < 0.01$

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 80 male and 80 female ICR (Crj:CD-1) mice were fed diets containing buprofezin (purity, 99.5%) at a concentration of 0, 20, 200, 2000 or 5000 ppm (equal to 0, 1.8, 17.4, 190 and 481 mg/kg bw per day in males and 0, 1.9, 17.9, 191 and 493 mg/kg bw per day in females) for 2 years (104 weeks). Ten males and ten females were killed at 52 weeks for evaluation of toxicity. In all mice, clinical signs of toxicity, body-weight gains, feed and water consumption were regularly recorded. Haematological, clinical-chemistry and urine parameters were investigated at 52 weeks and at study termination. At study termination, organ weights were measured and histology on selected organs was performed. A statement of quality assurance was given.

There were no treatment-related clinical signs or mortalities in any of the treated groups. In males and females at 5000 ppm, body weights were slightly (females, 5–10%; and males, approximately 5%) but statistically significantly reduced from week 6 (males) and week 9 (females) onwards. A very slight trend to reduced body weight was also observed at 2000 ppm in males and females. These effects were not more statistically significant by the end of the study (Table 19). No changes in feed consumption in any group were observed.

Urine analysis showed a minor but statistically significant decrease (1–2%) in specific gravity at 52 and 104 weeks in males and females. In females at 2000 and 5000 ppm, more mice showed low urinary ketone-body levels. Haematology showed that mice at the highest dose at 104 weeks had statistically significantly increased platelet numbers (males, 19%; and females, 25%). In females at the highest dose at 52 weeks, but not at 104 weeks, erythrocytes, erythrocyte volume fraction and haemoglobin concentrations were statistically significantly reduced by 5–8%. Females in the groups at 2000 ppm and 5000 ppm at 52 weeks and males at 5000 ppm at 104 weeks had statistically significantly increased cholesterol concentrations.

At 52 weeks, absolute and relative liver weights were increased in males at 200 ppm and above and in females at 2000 ppm and above (Table 19). At 104 weeks, liver-weight increases were statistically significant only in males at the highest dose. Absolute and relative adrenal weights were also increased

in males at 52 weeks, but not at 104 weeks. In males and females at the highest dose, cloudy and dark-coloured livers and in males atrophic/softened testis were found with increased incidences. The only clearly treatment-related non-neoplastic histological changes were hypertrophic and hyperplastic alterations in the livers of males and females at 2000 ppm and above (Table 20). In female mice, the incidence of liver adenoma at 2000 and 5000 ppm was increased above the historical mean but was within the range for historical controls (historical controls: mean, 5.4%; range, 1.3–12.5%), but

Table 19. Liver-weight changes[a] in a 2-year study in mice given diets containing buprofezin

Dietary concentration (ppm)	52 weeks			104 weeks		
	Body weight (g)	Absolute liver weight (g)	Relative liver weight (%)	Body weight (g)	Absolute liver weight (g)	Relative liver weight (%)
Males						
0	51.5 ± 5.1	2.38 ± 0.24	4.65 ± 0.53	47.5 ± 7.6	3.32 ± 1.57	7.30 ± 4.00
20	51.8 ± 3.4	2.48 ± 0.33	4.79 ± 0.57	48.7 ± 5.9	3.69 ± 2.07	7.44 ± 3.74
200	54.5 ± 4.4	2.91 ± 0.44**	5.36 ± 0.80*	48.4 ± 7.3	3.35 ± 1.43	7.08 ± 3.33
2000	50.9 ± 4.3	2.80 ± 0.40*	5.50 ± 0.70*	48.0 ± 4.8	3.73 ± 1.67	7.82 ± 3.39
5000	49.9 ± 4.2	3.34 ± 0.70**	6.64 ± 0.94***	46.5 ± 6.2	4.40 ± 2.24*	9.57 ± 4.02*
Females						
0	50.1 ± 7.2	1.90 ± 0.29	3.83 ± 0.63	48.2 ± 9.2	2.44 ± 1.14	5.36 ± 3.54
20	50.9 ± 7.4	2.05 ± 0.25	4.13 ± 1.06	46.0 ± 9.6	2.04 ± 0.52	4.57 ± 1.41
200	49.3 ± 6.8	1.95 ± 0.31	3.98 ± 0.57	46.5 ± 7.0	2.22 ± 0.61	4.87 ± 1.70
2000	49.1 ± 4.7	2.28 ± 0.31*	4.64 ± 0.39**	46.4 ± 6.3	2.79 ± 1.08	6.02 ± 2.11
5000	46.3 ± 6.1	2.52 ± 0.35***	5.48 ± 0.72***	44.5 ± 4.9	2.74 ± 0.60	6.20 ± 1.41

From Yoshida (1983)

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

[a] ± standard deviation

Table 20. Incidence of non-neoplastic and neoplastic liver lesions in a 2-year study in mice given diets containing buprofezin

Liver lesions	Dietary concentration (ppm)									
	Males (n = 80)					Females (n = 80)				
	0	20	200	2000	5000	0	20	200	2000	5000
Non-neoplastic										
Centrilobular hepatocellular swelling	3	3	0	10*	13**	3	4	3	15**	12*
Diffuse hepatocellular swelling	9	9	10	14	42**	2	2	1	1	24**
Hepatocellular hyperplasia	12	17	17	13	27**	2	5	7	9*	8*
Neoplastic										
Adenoma	13	12	16	11	17	2	2	1	7	8*
Carcinoma	14	11	11	18	15	3	2	0	4	4

From Yoshida (1983)

* $p < 0.05$

** $p < 0.01$

there was no dose-related increase in incidence between 2000 and 5000 ppm. In these two groups, the incidence of liver carcinoma was also increased and was greater than the historical mean and also slightly above the range for historical controls (historical controls: mean, 1.0%; range, 0–3.8%). However, the incidence of carcinoma in concurrent controls was also at the upper bound of the range for historical controls and therefore there was no dose–response relationship in this study.

The NOAEL for toxicity was 200 ppm, equal to 17.4 mg/kg bw per day, on the basis of transiently increased liver-weight gains and histological changes in the liver at 2000 ppm in males and females. The NOAEL for carcinogenicity was 5000 ppm, equal to 481 mg/kg bw per day, the highest dose tested (Yoshida, 1983).

Rats

Groups of 55 male and 55 female Jcl:SD rats were fed diets containing buprofezin (purity, > 99.0%) at a concentration of 0, 5, 20, 200 or 2000 ppm (equal to 0, 0.26, 0.90, 8.71 or 89.46 mg/kg bw per day in males and 0, 0.33, 1.12, 11.19 or 114.71 mg/kg bw per day in females) for 2 years (104 weeks). Five males and five females per group were killed at 26 weeks, and ten males and ten females per group were killed at 52 weeks. Clinical signs of toxicity, body-weight gains and feed consumption were recorded regularly. Haematological, clinical-chemistry and urine parameters were investigated at 26, 52 weeks and at study termination. Phenolsulfonphthalein (PSP, phenol red) excretion into the urine after intramuscular administration and bromosulfonphthalein (BSP) excretion into serum after intravenous administration were analysed in rats in the control group and in the group at the highest dose before study termination. At weeks 26 and 52 for the interim sacrifice groups and at study termination, organ weights were recorded and histological investigations on selected organs were performed. The study complied with GLP.

No treatment-related changes in clinical parameters or mortality were seen in any group receiving buprofezin. Body weight in males and females at the highest dose were slightly lower than in controls, statistically significant only in the first 5 weeks of treatment in males and throughout the study with the exception of the period from month 7 to 13 in females, respectively. In males, bw were lower by 2–4% and in females by 5–10%. Feed intake was not significantly affected by treatment in any group. No treatment-related changes in haematological parameters and PSP and BSP excretion were found. In clinical chemistry, statistically significant and dose related decreases in serum glucose levels were seen in females at 20 ppm and above but not in males or females at any other time-point. In males at 2000 ppm, ALT and AST activity were consistently lower by 25–30% than in control, the effect being statistically significant at 6 and 24 months. At terminal sacrifice, the livers of a few males and females had a dark brownish appearance. Absolute and relative liver weights were increased in males and females at the highest dose at all time-points. Absolute and relative thyroid weights were increased in females at the highest dose at 26 and 52 weeks and, although not statistically significantly, at study termination (Table 21). In males and females at 2000 ppm, increased incidences of hepatocellular hypertrophy were seen at all sacrifice time-points (Table 22). This effect was accompanied by proliferation of the smooth endoplasmic reticulum in hepatocytes. Focal hepatocellular necrosis was also increased at 52 and 104 weeks in females, and in males and females at 2000 ppm. More hyperplastic hepatocellular nodules were found in males and females at study termination. At 2000 ppm, males and females exhibited very high incidences of thickened and/or hyperplastic thyroid follicular epithelia. Increased incidences of cardiac-muscle necrosis were seen in males and females at 2000 ppm group at 52 weeks. At study termination, the incidence of this effect was high in all groups, including controls. No dose-related increases in tumour incidences were observed (Watanabe et al., 1982).

After a change in diagnostic criteria and histopathology terminology in the late 1980s, the histology slides of the 2-year feeding study in rats (Watanabe et al., 1982) were re-examined in the

early 1990s (Todhunter & Goodman, 1995). Particularly affected by this criteria change were the liver lesions previously termed "hyperplastic nodules". Under the new classification standards, some of these lesion were re-classified as "hepatocellular adenoma" or "foci of cellular alteration". The study complied with GLP.

At 2000 ppm, males and females showed increased incidences of centrilobular hepatocellular hypertrophy and females also showed a slightly higher incidence of adenoma and eosinophilic foci (Table 23). The increase in liver adenoma was statistically not significant, not found in males and not accompanied by an increase in liver carcinoma it was judged accidentally. With regard to the thyroid, F-cell hypertrophy was seen in males at 200 ppm and in males and females at 2000 ppm. C-cell hyperplasia was increased statistically significantly at 2000 ppm in males and females (Table 24)

The NOAEL was 20 ppm, equal to 0.9 mg/kg bw per day, on the basis of slight increases in the incidence of thyroid F-cell hypertrophy at 200 ppm.

2.4 Genotoxicity

The genotoxic potential of buprofezin was investigated in a battery of studies of genotoxicity in vitro and two studies of micronucleus formation in mice in vivo (Table 29). All studies in vitro gave clearly negative results with and without metabolic activation.

One out of two studies of micronucleus formation in mice gave equivocal results and one gave negative results. In the first study, groups of six male and female BDF1 mice were given buprofezin at a dose of 0, 6400, 8000 or 10 000 mg/kg bw by gavage. In a first experiment, bone-marrow smears were taken from mice at 0 or 10 000 mg/kg bw at 12, 48 or 72 h after dosing, while in a second experiment bone-marrow smears were taken from mice at 0, 6400 and the 8000 mg/kg bw at 24 h

Table 21. Relative liver and thyroid weights in a 2-year study in rats fed diets containing buprofezin

Dietary concentration (ppm)	Relative (to body weight) weight [%]					
	26 weeks		52 weeks		104 weeks	
	Liver	Thyroid[a]	Liver	Thyroid[a]	Liver	Thyroid[a]
Males						
0	2.59 ± 0.25	3.65 ± 1.13	2.43 ± 0.24	4.17 ± 1.11	2.69 ± 069	5.18 ± 2.26
5	2.52 ± 0.11	3.82 ± 0.44	2.28 ± 0.15	3.70 ± 1.14	2.71 ± 0.45	4.79 ± 1.25
20	2.83 ± 0.16	3.60 ± 1.89	2.41 ± 0.14	3.73 ± 0.97	2.83 ± 0.88	4.88 ± 1.23
200	2.52 ± 0.19	3.34 ± 0.59	2.57 ± 0.18	3.94 ± 0.58	2.74 ± 0.66	4.99 ± 1.71
2000	3.13 ± 0.17**	4.59 ± 0.71	2.99 ± 0.24**	4.71 ± 1.38	3.33 ± 0.93*	10.13 ± 11.60
Females						
0	2.48 ± 0.18	4.75 ± 1.04	2.47 ± 0.17	5.20 ± 0.67	2.58 ± 0.43	5.52 ± 2.88
5	2.66 ± 0.34	5.02 ± 0.84	2.57 ± 0.29	5.04 ± 1.14	2.43 ± 0.27	4.75 ± 1.58
20	2.55 ± 0.17	5.67 ± 0.89	2.45 ± 0.16	5.43 ± 1.15	2.63 ± 0.45	5.41 ± 1.17
200	2.51 ± 0.13	5.14 ± 1.55	2.52 ± 0.18	6.20 ± 1.43	2.71 ± 0.35	5.13 ± 1.31
2000	3.16 ± 0.22**	8.06 ± 1.53**	3.31 ± 0.34**	6.69 ± 1.63*	3.22 ± 0.61**	6.97 ± 2.29

[a] × 1000

From Watanabe et al. (1982)

* $p < 0.05$

** $p < 0.01$

Table 22. Incidence of non-neoplastic lesions in a 2-year study in rats fed diets containing buprofezin

Non-neoplastic lesion	Incidence of lesion									
	Dietary concentration (ppm)									
	Males					Females				
	0	5	20	200	2000	0	5	20	200	2000
26 weeks										
No. of rats examined	5	5	5	5	5	5	5	5	5	5
Enlargement of hepatocytes	0	0	0	0	2	0	0	0	0	3
Thickening and/or hyperplasia of thyroid follicular epithelium	0	0	0	1	5	0	0	0	1	5
Necrosis of cardiac muscle	1	0	1	0	0	1	0	0	1	0
52 weeks										
No. of rats examined	10	10	10	10	10	10	10	10	9	10
Enlargement of hepatocytes	0	0	0	0	4	2	0	0	0	10
Focal necrosis of hepatocytes	0	1	0	1	1	0	0	0	2	4
Enlargement of hepatocellular nuclei	0	0	1	1	0	0	0	0	0	8
Thickening and/or hyperplasia of thyroid follicular epithelium	0	0	0	2	8	0	0	0	0	10
Necrosis of cardiac muscle	1	1	2	0	3	0	0	0	1	4
104 weeks										
No. of rats examined	20	14	19	17	24	19	18	18	15	13
Enlargement of hepatocytes	1	0	0	0	7	0	0	0	0	2
Focal necrosis of hepatocytes	1	3	7	0	6	2	1	5	2	4
Enlargement of hepatocellular nuclei	5	6	8	4	6	6	6	6	6	6
Hyperplastic nodules of hepatocytes	1	1	4	0	7	0	2	2	3	5
Thickening and/or hyperplasia of thyroid follicular epithelium	0	1	0	1	24	0	0	0	0	9
Proliferation of parafollicular cells	2	1	0	3	19	0	0	0	0	10
Necrosis of cardiac muscle	11	9	15	5	19	7	11	14	10	7

From Watanabe et al. (1982)

only. In a confirmatory experiment, bone-marrow smears were taken from mice at 0, 6400, 8000 or 10 000 mg/kg bw at 24 h after dosing. In a further experiment, groups of male and female mice received four doses of buprofezin at 0 or 10 000 mg/kg bw on four consecutive days and bone-marrow smears were taken 12, 24, 48 and 72 h after administration of the last dose.

The ratio of polychromatic erythrocytes (PCE) to total erythrocytes (TE) was only slightly decreased in the experiment with repeated doses. Therefore, buprofezin was not excessively toxic to the bone marrow.

A statistically significant increase in the frequency of micronucleated PCE (MN-PCE) was found in all males receiving buprofezin and in females at 10000 mg/kg bw in the first and second experiments (Table 26). In the confirmatory experiment and in the experiment with repeated doses for 4 days, no evidence for micronucleus formation was identified. In view of the minor increase in the frequency of MN-PCE and its non-reproducibility, the Meeting concluded that this finding was not treatment-related (Sasaki, 1983).

Table 23. Incidence of liver lesions at study termination in a 2-year study in rats fed diets containing buprofezin, as originally classified and after re-examination

Liver lesion	Incidence of liver lesions (%)									
	Dietary concentration (ppm)									
	Original report					Re-examination				
	0	5	20	200	2000	0	5	20	200	2000
Males										
No. of rats examined	40	40	40	40	40	39	37	39	40	40
Adenoma	0.0	0.0	0.0	0.0	0.0	2.6	2.7	7.7	0.0	10.0
Carcinoma	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	2.5
Adenoma/carcinoma combined	0.0	0.0	0.0	0.0	0.0	5.1	2.7	7.7	0.0	12.5
Hyperplastic nodules	5.0	2.5	12.5	2.5	17.5*	—	—	—	—	—
Hyperplastic foci	20.0	25.0	25.0	25.0	25.0	—	—	—	—	—
Centrilobular hypertrophy	—	—	—	—	—	0.0	0.0	0.0	0.0	27.5*
Diffuse hypertrophy	—	—	—	—	—	5.1	5.4	7.7	5.0	17.5*
Eosinophilic foci	—	—	—	—	—	20.5	13.5	12.8	15.0	17.5
Homogenous basophilic foci	—	—	—	—	—	2.5	5.4	12.8	2.5	0.0
Tigroid basophilic foci	—	—	—	—	—	7.7	24.3	7.7	2.5	10.0
Females										
No. of rats examined	39	40	40	40	40	39	39	40	40	39
Adenoma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.7
Carcinoma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adenoma/carcinoma combined	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.7
Hyperplastic nodules	0.0	7.5	5.0	10.0*	15.0*	—	—	—	—	—
Hyperplastic foci	15.4	25.0	15.0	17.5	27.5*	—	—	—	—	—
Centrilobular hypertrophy	—	—	—	—	—	0.0	0.0	0.0	0.0	35.9*
Diffuse hypertrophy	—	—	—	—	—	12.8	2.6	7.5	10.0	15.4*
Eosinophilic foci	—	—	—	—	—	10.3	10.3	12.5	22.5	28.2
Homogenous basophilic foci	—	—	—	—	—	2.5	5.1	5.0	7.5	0.0
Tigroid basophilic foci	—	—	—	—	—	0.0	2.5	10.0	15.0*	5.1

From Watanabe et al. (1982); Todhunter & Goodman (1995)

* $p < 0.05$

Table 24. Incidence of thyroid lesions in rats fed diets containing buprofezin, according to the re-examination report at termination in a 2-year study

Thyroid lesion	Incidence of lesion (%)									
	Dietary concentration (ppm)									
	Males					Females				
	0	5	20	200	2000	0	5	20	200	2000
No. of rats examined	36	35	38	39	39	37	36	40	33	39
F-cell adenoma	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	2.6
F-cell carcinoma	0.0	0.0	0.0	2.6	0.0	2.7	0.0	0.0	0.0	2.6
Adenoma/carcinoma combined	0.0	0.0	0.0	2.6	2.6	2.7	0.0	0.0	0.0	5.1
F-cell hypertrophy	16.7	31.4	31.6	48.7*	64.1*	8.1	5.6	2.5	12.1	51.3*
F-cell hyperplasia	0.0	0.0	0.0	0.0	5.1	0.0	0.0	0.0	0.0	2.6
C-cell adenoma	8.3	5.7	5.3	2.6	0.0	5.4	2.8	0.0	3.0	0.0
C-cell carcinoma	0.0	0.0	2.6	2.6	5.1	0.0	0.0	0.0	0.0	0.0
Adenoma/carcinoma combined	8.3	5.7	7.9	5.1	5.1	5.4	2.8	0.0	3.0	0.0
C-cell hyperplasia	31.1	62.9	73.7	64.1	84.6*	59.5	55.6	60.0	66.7	82.1*

From Todhunter & Goodman (1995)

* $p < 0.05$

Table 25. Incidences of thyroid lesions in rats fed diets containing buprofezin, according to the original report at termination in 2-year study

Thyroid lesion	Incidence of lesion (%)									
	Dietary concentration (ppm)									
	Males					Females				
	0	5	20	200	2000	0	5	20	200	2000
No. of rats examined	40	40	40	40	39	39	39	40	40	40
Follicular epithelial thickening/hyperplasia	2.5	2.5	0.0	7.5	82*	0.0	0.0	0.0	2.5	43*

From Watanabe et al. (1982)

* $p < 0.05$

In the second study, groups of five male Slc/ICR mice were given two doses of buprofezin at 0, 500, 1000 or 2000 mg/kg bw by gavage on two consecutive days. Mitomycin and colchicine were used as positive controls for substances known to provoke micronucleation. In a confirmatory experiment, mice were given two doses of buprofezin at 0 or 2000 mg/kg bw by gavage on two consecutive days. Bone-marrow smears were prepared for staining with acridine orange 24 h after the last dose. Immature erythrocytes and micronuclei were identified as red and yellowish green fluorescence-emitting bodies, respectively. In a further experiment, the mechanism of DNA damage was investigated with an anti-kinetochore antibody (CREST) and DNA-propidium staining .

The ratio of immature erythrocytes to total erythrocytes was only slightly decreased at the highest dose in both experiments. The Meeting therefore concluded that buprofezin was not excessively toxic to the bone marrow. In both experiments, a statistically significant increase in micronucleated

Table 26. The frequency of micronucleated polychromatic erythrocytes as a percentage of polychromatic erythrocytes in bone-marrow smears from BDF1 mice given buprofezin by gavage

Dose (mg/kg bw)	Frequency of micronucleated polychromatic erythrocytes (% of polychromatic erythrocytes)							
	Time after dosing (h)							
	12	24	48	72	12	24	48	72
	Males				Females			
Single dose: first experiment								
0	0.07	0.12	0.23	0.18	0.17	0.10	0.15	0.18
10 000	0.18	0.27*	0.23	0.10	0.08	0.30*	0.20	0.10
Single dose: second experiment								
0	—	0.03	—	—	—	0.10	—	—
6 400	—	0.15*	—	—	—	0.13	—	—
8000	—	0.20**	—	—	—	0.08	—	—
Single dose: confirmatory experiment								
0	—	0.10	—	—	—	0.02	—	—
6 400	—	0.07	—	—	—	0.03	—	—
8 000	—	0.17	—	—	—	0.03	—	—
10 000	—	0.03	—	—	—	0.05	—	—
Repeated doses								
0	0.18	0.15	0.10	0.08	0.10	0.08	0.12	0.07
10 000	0.26	0.15	0.14	0.01	0.23	0.16	0.14	0.15

From Sasaki (1983)

* $p < 0.05$

** $p < 0.01$

Table 27. Frequency of micronucleated immature erythrocytes and kinetochore-positive micronuclei in Slc/ICR mice given two doses of buprofezin by gavage

Test substance	Dose (mg/kg bw)	Micronucleated immature erythrocytes (% of immature erythrocytes)		Kinetochore-positive micronuclei (% of all micronuclei)
		First experiment	Confirmatory experiment	
Buprofezin	0	0.15	0.11	24.0
	500	0.12	—	—
	1000	0.18	—	—
	2000	2.02**	0.34**	31.6
Mitomycin	3	6.95**	4.65**	14.6*
Colchicine	1	—	1.90**	61.2**

From Inagaki (2006c)

* $p < 0.05$

** $p < 0.01$

immature erythrocytes was observed at the highest dose. While the aneugenic substance colchicine significantly increased the percentage of the kinetochore-positive micronuclei, the highest dose of buprofezin showed a slight and statistically not significant increase in kinetochore-positive micronuclei (Inagaki, 2006c).

In a published study, Syrian hamster embryo cells were exposed to buprofezin at a concentration of 12.5–100 μmol/l and the induction of morphological transformation of colonies, DNA repair-synthesis and micronuclei were investigated. To separate a putative aneugenic potential from clastogenic potential, kinetochore-positive micronuclei were stained with an anti-kinetochore serum (CREST). As control substances for aneugenic or clastogenic effects, respectively, the known aneugenic compound diethylstilbestrol (DES) and the clastogenic compound nitroquinoline-*N*-oxide (NQO) were included. As control substance for morphological transformation of Syrian hamster embryo cell colonies, praziquantel (PRZ) was used. The cloning efficiency and survival of cells treated with buprofezin were slightly lowered at all doses in a non-dose-related manner. Treatment with buprofezin did not induce DNA repair-synthesis at any dose. A clear response to treatment with buprofezin was seen in the form of increased transformation frequency, increase in micronuclei and kinetochore-positive micronuclei was seen at the highest concentration of 100 μmol/l, equal to 30.5 μg/ml (Table 28) (Herrera et al., 1993).

In another published study, buprofezin was tested for its potential to induce chromosomal aberrations in male mice given a single oral dose of a 25% formulation. At doses of 1.4 and 2.8 g/kg yes bw (corresponding to active ingredient, 350 and 700 mg/kg bw), an increase in the frequency of abnormal metaphase chromosomes of up to twofold was seen. Because the test material was a formulation with unknown ingredients, the increase in metaphase abnormalities was not interpretable because it was not necessarily related to treatment with buprofezin (Fahmy & Abdalla, 1998).

In conclusion, buprofezin was tested for genotoxicity in an adequate range of studies in vitro and for micronucleus formation in vivo. In the submittestudies, there was no evidence for genotoxicity in vitro; however, in the published non-GLP study (Herrera et al., 1993), micronuclei were induced in cultured cells by an aneugenic mechanism, rather than by chromosomal breakage.

Table 28. Frequency of micronucleated immature erythrocytes and kinetochore-positive micronuclei in Syrian hamster embryo cells treated with buprofezin

Test substance	Concentration (μmol/l)	Transformation frequency (%)	Micronuclei per 2000 cells[a]	Kinetochore-positive micronuclei (%)
DMSO	0.1%	< 0.12	22.0 ± 4.6	12
Buprofezin	12.5	0.17	29.7 ± 6.7	57
	25	0.19	32.0 ± 6.6*	58
	50	0.18	31.3 ± 1.5**	50
	100	0.80	51.3 ± 5.5***	70
PRZ	50	0.18	—	—
NQO	1	—	64.7 ± 12.7***	22
DES	30	—	—	69

From Herrera et al. (1993)

DES, diethylstilbestrol; DMSO, dimethyl sulfoxide; NQO, nitroquinoline-*N*-oxide; PRZ, praziquantel

[a] Three independent experiments

* $p < 0.05$

** $p < 0.025$

** $p < 0.005$

Table 29. Results of studies of genotoxicity with buprofezin

End-point	Test system[a]	Concentration	Purity (%)	GLP	Result	Reference
In vitro						
Reverse mutation[b]	*S. typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100 [c]	1.6–5000 µg/ml ± S9	99.8	Yes	Negative ± S9	Callander (1988)
Reverse mutation	*S. typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100 and *E. coli* (WP2 *hcr*)	10–5000 µg/ml ± S9	99.3	Not stated	Negative ± S9	Moriya (1980)
DNA damage	*Bacillus subtilis* (H17 and M45*rec*)	20–5000 µg/disc	99.3	Not stated	Negative	Moriya (1980)
Chromosome aberrations [d]	Chinese hamster lung cells (CHL); 6 h exposure [c]	64.1–77.9 µg/ml + S9 26.5–38.2 µg/ml – S9	99.9	Yes	Negative ± S9	Inagaki (2006a)
Forward mutation[b]	Mouse lymphoma cell line L5178 ($TK^{+/-}$)	17.8–100 µg/ml + S9 13.3–42.2 µg/ml – S9	99.8	Yes	Negative ± S9	Cross (1988)
DNA damage	Human lymphocytes	10–100 µg/ml ± S9	99.8	Yes	Negative ± S9	Howard & Richardson (1988)
Unscheduled DNA synthesis	Primary rat hepatocytes	10^{-2}–10 µmol/l	99.8	Yes	Negative	Trueman (1988)
In vivo						
Micronucleus formation	BDF1 mice	Single dose at 6400, 8000, or 10 000 mg/kg bw and four consecutive doses at 10 000 mg/kg bw	99.5	Not stated	Negative	Sasaki (1983)
Micronucleus formation	Slc/ICR mice (males)	Two doses at 500, 1000, or 2000 mg/kg bw for two consecutive days	99.9	Yes	Equivocal	Inagaki (2006c)

S9, 9000 × *g* supernatant from rat livers.

[a] Positive controls were included in all tests for genotoxicity.

[b] Tests were conducted in duplicate.

[c] Precipitation at the highest concentration.

[d] Negative results also after exposure over 1.5–3.0 cell cycle lengths –S9 at 7.73–21.4 µg/ml.

In the experiments on micronucleus induction in immature erythrocytes of bone marrow of mice in vivo, conflicting results were obtained. One study reported statistically significantly increased incidences in two experiments, but the numerical results were very different and were nd ot fully supported by the equivocal results of an earlier study in which the doses administered were five times higher. Furthermore, the suggestion of an aneugenic effect in vitro in the published study was not confirmed in vivo.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 30 male and 30 female Wistar-Imachi rats (age 3 weeks) were fed diets containing buprofezin (purity, 99.1%) at a concentration of 0, 10, 100 or 1000 ppm, equal to 0, 0.6–0.8, 6.0–8.1 or 62.5–86.5 mg/kg bw per day in males and 0, 0.8–0.9, 7.9–8.9 or 82.6–85.2 mg/kg bw per day in females. All rats were exposed throughout the study, beginning 13 weeks before mating. After mating, dams were allowed to produce two litters (F_1a and F_1b). For developmental investigations, ten dams per group underwent caesarian section on day 21 of gestation. To produce F_1 parents, the remaining dams were allowed to rear F_1b pups. F_2a and F_2b generations were treated with buprofezin in the same way as the F_1a and F_1b generations. Feed and water intake and body weights were recorded weekly and clinical signs were recorded daily. The reproductive parameters fertility, duration of gestation, litter size, live and dead pups and postnatal development of pups were recorded. Upon caesarian section, implantation and pup developmental parameters were investigated. All rats in this study underwent a gross pathology examination. A statement of quality assurance was provided.

No clinical signs of toxicity attributable to treatment with burofezin were observed at any dose. F_1 and F_2 but not F_0 males and females at 10, 100 or 1000 ppm had statistically significantly lowered body weight in the first weeks of treatment (up to approximately 17% in males at the highest dose and approximately 10% in females, respectively). Only in males at 1000 ppm did this effect persist throughout the study, although with a clear trend for recovery. At all other doses, the low body weights returned to normal values within 2–6 weeks. F_0 and F_1 females had also slightly lower body weights at days 0 or 4 of lactation at all doses. Thereafter, body weights increased to normal. Feed and water intake was not affected by treatment.

Fertility parameters were not affected in males or females receiving buprofezin. The viability index at postnatal day 4 was lower in the F_1a generation at 10 and 1000 ppm, but comparable to values for controls in the other generations. The body-weight gains in male and female F_1a, F_1b, F_2a and F_2b pups decreased statistically significantly from postnatal day 4 to postnatal day 21 in the group at 1000 ppm when compared with values for the controls. At postnatal day 21, mean group body weights in this group were 10–18% lower than values for the controls**.** The incidences of skeletal and visceral variations and malformations were similar in all groups. With the exception of F_0 males, absolute and relative liver weights were increased in males and females at the highest dose in the F_0, F_1 and F_2 generations.

The NOAEL for parental toxicity was 100 ppm, equal to 6.0–8.1 mg/kg bw per day, on the basis of decreased body-weight gain in males at 1000 ppm. The NOAEL for reproductive effects was 1000 ppm, equal to 62.5–86.5 mg/kg bw per day, the highest dose tested. The NOAEL for pup development was 100 ppm, equal to 6.0–8.1 mg/kg bw per day, on the basis of reduced pup body-weight gain during lactation at 1000 ppm (Takeshima, 1982).

In a supplemental study, groups of 25 male and 25 female Wistar-Imachi rats (age 3 weeks) were fed diets containing buprofezin (purity, 99.1%) at a concentration of 0, 10, 100 or 1000 ppm,

equal to 0, 0.6, 6.4 or 65.6 mg/kg bw per day in males and 0, 0.9, 8.9 or 82.8 mg/kg bw per day in females All rats were exposed throughout the study, beginning 13 weeks before mating. The design of this study was the same as that of the previous study (Takeshima, 1982), except that only one generation (F_1a and F_1b) was produced and no caesarian sections for fetal examinations were performed.

No treatment-related effects on body-weight development, feed intake, clinical condition or fertility were observed. As in the first study, body-weight gains of male and female F_1a and F_1b pups decreased statistically significantly from postnatal day 4 to postnatal day 21 in the group at 1000 ppm when compared with values for the controls. At postnatal day 21, mean group body weights were 12–17% lower than those of the controls. Relative liver weights in male and females of the F_0 generation were slightly increased.

The NOAEL for parental and reproductive effects was 1000 ppm, equal to 65.6 mg/kg bw per day, the highest dose tested. The NOAEL for pup development was 100 ppm, equal to 6.4 mg/kg bw per day, on the basis of reduced pup body-weight gain during lactation at 1000 ppm (Takeshima, 1985).

In a third study of reproductive toxicity, groups of 26 male and 26 female Wistar-Imachi rats (age 3 weeks) were fed diets containing buprofezin (purity, 99.0%) at a concentration of 0, 10, 100 or 1000 ppm, equal to 0, 0.64–0.75, 6.46–7.42 or 66.0–73.97 mg/kg bw per day in males and 0, 0.92–1.02, 9.21–10.2 or 93.11–99.57 mg/kg bw per day in females. All rats were exposed throughout the study, beginning 10 weeks before mating. Only one filial generation in F_1 and F_2 were produced. Feed intake and bodyweights were recorded weekly and clinical signs were recorded daily. Fertility, duration of gestation, litter size, live and dead pups and postnatal development of pups were recorded. All rats underwent a gross pathology examination and selected organs were examined histologically. The study complied with GLP.

There were no treatment-related effects on clinical parameters, body weights, feed intake, fertility or litter data at any dose. Pup body-weight gains of males and females decreased statistically significantly from postnatal day 7 to postnatal day 21 in the group at 1000 ppm. At postnatal day 21, mean group body weights were approximately 10% lower in rats at 1000 ppm than in the control group. Statistically significant increases in absolute and relative organ weights at 1000 ppm were found in the kidney of males of both generations (Table 30). Other organ-weight changes in males and females were either only significant as absolute or relative weights or found in one generation only and therefore judged not to be relevant. There were no histological correlates accompanying the slight organ-weight changes.

The NOAEL for parental toxicity was 100 ppm, equal to 6.46 mg/kg bw per day, on the basis of very slight organ-weight changes. The NOAEL for reproductive effects was 1000 ppm, equal to 66.0 mg/kg bw per day, the highest dose tested. The NOAEL for offspring toxicity was 100 ppm, equal to 6.46 mg/kg bw per day, on the basis of reduced pup body-weight gain at 1000 ppm during lactation (Toyohara, 1997).

(b) Developmental toxicity

Rats

Groups of 22 one-to-one paired and pregnant Sprague Dawley CD rats received buprofezin (purity, 99.0%) at a dose of 0, 50, 200 or 800 mg/kg bw per day in 2% gum arabic by oral gavage on days 6–15 of gestation. The rats were examined daily for clinical condition and mortalities, and body weight, feed intake and water consumption were recorded regularly. On day 20 of gestation, fetuses were delivered by caesarean section and uterine examination was performed. Fetuses were examined for external, skeletal and visceral abnormalities. The study complied with GLP.

Table 30. Organ weight changes in parental rats given diets containing buprofezin in a study of reproductive toxicity

Organ	Weight	Organ-weight change (% of values for controls)							
		Males				Females			
		0	10	100	1000	0	10	100	1000
F_0 generation									
Liver	Absolute	100.0	110.6*	103.8	114.9**	100.0	96.0	98.3	111.3**
	Relative	100.0	106.5*	101.8	110.6**	100.0	94.4	95.1	106.9
Kidney	Absolute	100.0	107.1*	105.6	111.7**	100.0	107.5**	104.5	104.1
	Relative	100.0	103.6	103.9	108.2**	100.0	105.7**	101.3	99.7
Adrenals	Absolute	100.0	109.6*	101.9	109.6*	100.0	101.6	106.3	115.6**
	Relative	100.0	107.1	100.0	107.1	100.0	99.5	103.2	110.4*
F_1 generation									
Liver	Absolute	100.0	107.4*	104.2	107.9*	100.0	98.4	95.1	102.2
	Relative	100.0	103.6	99.8	103.4	100.0	99.7	93.7	98.8
Kidney	Absolute	100.0	106.8**	108.6**	110.2**	100.0	100.7	98.2	94.7
	Relative	100.0	102.8	103.9	105.7**	100.0	102.4	96.8	92.2*
Adrenals	Absolute	100.0	105.1	105.1	111.9**	100.0	98.7	98.7	107.9*
	Relative	100.0	101.8	100.9	108.0	100.0	100.8	97.9	105.0

From Toyohara (1997)

* $p < 0.05$

** $p < 0.01$

Thirteen rats at the highest dose showed several signs of ill-health: loose faeces, lethargy, hunched posture, thin appearance, piloerection and partially closed eyelids. The first appearance of these effects was on day 10 of gestation. One rat in this group was killed in extremis with gastrointestinal disturbances. No treatment-related clinical signs were seen in rats at the other doses.

From day 7 to day 10 of gestation, females at the highest dose did not gain body weight and thereafter they gained body-weight at approximately at the same rate as in the other groups but remained consistently 8–9% lower in weight than the controls (Table 31). From day 7 of gestation onwards, feed intake was statistically significantly reduced and water intake increased in the group at the highest dose. Increased water consumption was also observed in rats at 200 mg/kg bw. On final necropsy, two dams in the group at the highest dose (but none in the other groups) had bilateral hydroureter. No other treatment-related changes were observed in dams.

Four dams in the group at the highest dose had total litter losses, presumably as early postimplantation losses. If these rats were not included, the postimplantation losses were still high and fetal body weights were statistically significantly low when compared with those of the controls (Table 32). In the group at the highest dose, there were more fetuses with a space between body-wall and organs, subcutaneous oedema and retarded ossification (Table 33). The incidence of retarded ossification was also slightly increased at 200 mg/kg bw per day.

The NOAEL for both maternal and fetal toxicity was 200 mg/kg bw per day on the basis of decreased body-weight gains in dams, total litter losses and delayed fetal development as shown by retarded ossification and low fetal body weight at 800 mg/kg bw per day (Tesh et al., 1987).

Table 31. Group mean body weights during gestation in a study of developmental toxicity in rats given buprofezin by gavage

Dose (mg/kg bw per day)	Mean body weights (g)											
	Day of gestation											
	0	7	8	9	10	11	12	14	15	16	18	20
0	211	244	250	254	260	267	272	285	294	305	334	363
50	211	248	251	256	261	268	273	286	297	309	337	366
200	212	248	253	259	266	272	278	290	298	308	332	362
800	211	245	245*	245*	244*	249*	254*	267*	274*	283*	304*	331*

From Tesh et al. (1987)

* $p < 0.001$

Table 32. Litter data for dams killed on day 20 of gestation in a study of developmental toxicity in rats given buprofezin by gavage

Dose (mg/kg bw per day)	No. of pregnant rats	Total litter losses	No. of live fetuses	No. of resorptions	Implantation losses (%)		Fetal weight (g)
					Pre-	Post-	
0	22	0	13.9 ± 1.7	0.7 ± 0.9	7.0	5.0	3.40 ± 0.06
50	22	0	14.1 ± 2.1	0.9 ± 0.9	7.6	5.8	3.41 ± 0.06
200	21	0	13.7 ± 2.1	1.0 ± 1.0	6.1	7.1	3.34 ± 0.06
800	21	4*	10.5 ± 5.7*	4.5 ± 2.1**	9.9 [a]	30.2**	—
800 [b]	17	0	12.9 ± 2.5	2.2 ± 1.5	10.1	14.7*	3.04 ± 0.07***

From Tesh et al. (1987)

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

[a] Derived from 18 litters.

[b] Derived from surviving rats bearing live fetuses.

Table 33. Incidence of fetal findings in a study of developmental toxicity in rats given buprofezin by gavage

Dose (mg/kg bw per day)	No. of litters examined	No. of fetuses	Incidence of finding (%)				
			Body-wall organ space	Subcutaneous oedema	Incomplete ossification		First thoracic vertebral centrum absent
					Interparietal bone	Sternebrae (third)	
0	22	153	1.3	15.1	39.9	5.2	1.3
50	22	159	0.7	9.9	42.1	6.9	1.3
200	21	143	2.8	21.5	60.8**[b]	14.0	0.7
800	17	112	18.5*[b]	45.4**[a]	74.1***[b]	25.9***[a]	10.7*[a]

From Tesh et al. (1987)

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

[a] Outside range for historical controls.

[b] Within range for historical controls.

Rabbits

Groups of 17 artificially inseminated and pregnant New Zealand White rabbits received buprofezin (purity, 99.0%) at a dose of 0, 10, 50 or 250 mg/kg bw per day in 2% gum arabic by oral gavage on days 6–19 of gestation. The rabbits were examined daily for clinical condition and mortalities, and body weight, feed intake and water consumption were recorded regularly. On day 29 of gestation, fetuses were delivered by caesarean section and uterine examination was performed. Fetuses were examined for external, skeletal and visceral abnormalities. The study complied with GLP.

In the group at 10 mg/kg bw per day, one rabbit died and another was killed in extremis. The reasons were not treatment-related gastrointestinal and respiratory tract disturbances. In the first 4 days of treatment, rabbits at the highest dose lost weight; thereafter they gained body weight at approximately at the same rate as did rabbits in the other groups but remained consistently 4–7% lighter than the controls (Table 34). In this group, feed intake on days 6–12 of gestation was approximately 35% lower than that of the controls; thereafter it increased to more than that of the controls. Water intake was not affected.

One rabbit at 50 mg/kg bw per day aborted on day 25 of gestation and two rabbits at 250 mg/kg bw per day showed total litter losses. Since this was within the range for historical controls (two litter losses in 1 out of 28 historical control groups, and one litter loss in 3 out of 28 historical control groups) a link with treatment is questionable. No other litter parameters were affected by treatment (Table 35). One fetus in rabbits at the intermediate dose and one in rabbits at the highest dose had agenesis of one kidney. A supplemental position paper provided by the company including additional historical data shows that this finding is occasionally induced by untreated carrier males (Yoshizane, 2008). Two fetuses in the group at the highest dose had enlarged aortic arches. No other dose-related effects on fetuses were observed (Table 36).

The NOAEL for maternal toxicity was 50 mg/kg bw per day on the basis of reduced bodyweight gain at 250 mg/kg bw per day. The NOAEL for fetal toxicity was 50 mg/kg bw per day on the basis of an increased incidence of enlarged aortic arches in fetuses at 250 mg/kg bw per day (Tesh et al., 1986).

2.6 Special studies

(a) Thyroid function

In rats, the thyroids of males and females given buprofezin at dietary concentrations of 1000 ppm and above in a 90-day feeding study (Watanabe, 1986) and at 2000 ppm in a 104-week feeding study (Watanabe et al., 1982; Todhunter & Goodman, 1995) showed thickening and hyperplasia of follicular epithelial cells. In a mechanistic study to elucidate the possible thyroidal mode of action of buprofezin, male SD rats, ddY mice, Hartley guinea-pigs and Japanese White rabbits were exposed to buprofezin (purity, 99%) and to propylthiouracil (PTU), which served as an anti-thyroidal positive control. In the animals tested, serum concentrations of tri-iodothyronine (T3) and thyroxin (T4) and protein-bound iodine (PBI) were measured. Additionally, thyroid weights were recorded and thyroid peroxidase (TP) activity was measured and pituitaries were investigated histologically. The number of animals used in the study was not given.

In test 1, groups of rats were given buprofezin at a dose of 0 or 500 mg/kg bw per day by gavage for 1, 2, 4, or 7 consecutive days; the control group was probably treated only once. In test 2, groups of rats were given buprofezin at a dose of 0, 100, 300, 500, 1000 mg/kg bw per day by gavage for 7 consecutive days.

Serum T3 concentrations were decreased after the administration of four doses of buprofezin at 500 mg/kg bw and T4 was decreased after two doses (Table 37). T3 and T4 concentrations were decreased after dosing at 300 mg/kg bw per day for 7 days (Table 38), although the decrease in T3 did not follow a dose–response relationship.

Table 34. Group mean body weights during gestation in a study of developmental toxicity in rabbits given buprofezin by gavage

Dose (mg/kg bw per day)	Mean body weight (kg)										
	Day of gestation										
	0	6	8	10	12	14	16	18	20	24	28
0	4.00	4.12	4.13	4.19	4.21	4.28	4.35	4.36	4.37	4.44	4.47
10	3.96	4.10	4.11	4.16	4.19	4.26	4.31	4.32	4.34	4.40	4.45
50	3.96	4.09	4.12	4.16	4.19	4.25	4.31	4.31	4.32	4.42	4.43
250	3.97	4.06	3.91	3.90	3.94	4.04	4.10	4.13	4.15	4.28	4.28

From Tesh et al. (1986)

Table 35. Litter data for dams killed on day 29 of gestation in a study of developmental toxicity in rabbits given buprofezin by gavage

Dose (mg/kg bw per day)	No. of pregnant animals	Abortion and total litter resorption	No. of live fetuses	No. of resorptions	Implantation losses (%)		Fetal weight (g)
					Pre-	Post-	
0	16	0	9.0 ± 2.0	0.6 ± 0.8	13.6	5.9	42.0 ± 1.8
10	14	0	7.9 ± 2.6	0.4 ± 0.6	19.0	4.3	44.0 ± 1.8
50	17	1[a]	8.5 ± 1.7	1.0 ± 1.0	12.1	10.5	41.8 ± 1.9
250	14	2[b]	7.8 ± 2.2	0.6 ± 0.8	14.4	6.9	41.2 ± 2.0

From Tesh et al. (1986)

[a] Abortion.

[b] Total litter resorption.

Table 36. Incidences of fetal observations in a study of developmental toxicity in rabbits given buprofezin by gavage

Fetal observation	Dose (mg/kg bw per day)				Historical controls[b]	
	0	10	50	250		
					Mean (%)	Range (%)
No. of fetuses examined	144	110	136	94		
Litters	16	14	16	12		
Aortic arch enlarged	0 (0%)	0 (0%)	0 (0%)	2 (2.1%)[a]	0.01	0–0.9
Agenesis of one kidney	0 (0%)	0 (0%)	1 (0.7%)	1 (1.1%)	0.02	0–1.1

From Tesh et al. (1986)

[a] Two litters affected.

[b] Data for 92 studies, 9385 fetuses.

Table 37. Serum concentrations of T3 and T4 in rats given buprofezin at 0 or 500 mg/kg bw per day for up to 7 days (test 1)

Day of treatment	Concentration (ng/dl)	
	T3	T4
Negative control[a]	99.0 ± 19.7	3.80 ± 1.33
1	110.4 ± 7.5	2.40 ± 0.30
2	84.6 ± 16.9	1.50 ± 0.41
4	65.6 ± 11.3	0.85 ± 0.65
7	60.6 ± 5.9	0.58 ± 0.37

From Konaka & Nokata (1989)

T3, triiodothyronine; T4, thyroxin.

[a] Probably treated once only at 0 mg/kg bw per day.

Table 38. Serum concentrations of T3 and T4 in rats given buprofezin at varying doses for 7 days (test 2)

Dose (mg/kg bw per day)	Concentration (ng/dl)	
	T3	T4
0	99.0 ± 12.6	2.86 ± 0.57
100	90.2 ± 10.7	2.54 ± 0.70
300	65.8 ± 7.6	1.16 ± 0.42
500	60.0 ± 16.9	0.41 ± 0.26
1000	68.8 ± 13.1	0.20 ± 0.23

From Konaka & Nokata (1989)

T3, triiodothyronine; T4, thyroxin.

Table 39. Serum concentrations of T3 and T4 in rats given diets containing buprofezin at varying doses or PTU for 1, 3 or 6 months (test 3)

Test substance	Dietary concentration (ppm)	Months of treatment		
		1	3	6
Concentration of T3 (ng/dl)				
Buprofezin	0	88.8 ± 19.1	114.8 ± 33.4	116.2 ± 14.1
	1000	79.0 ± 16.1	127.1 ± 25.1	120.0 ± 21.4
	5000	56.9 ± 11.1	138.4 ± 37.9	122.0 ± 6.3
PTU	200	17.0 ± 10.8	29.9 ± 11.8	20.5 ± 14.1
Concentration of T4 (µg/dl)				
Buprofezin	0	4.01 ± 0.62	4.46 ± 0.91	2.99 ± 0.63
	1000	3.93 ± 0.51	3.98 ± 0.43	2.90 ± 0.53
	5000	1.53 ± 0.51	2.43 ± 0.94	2.51 ± 0.03
PTU	200	ND	ND	0.51 ± 0.17

From Konaka & Nokata (1989)

ND, not detected; PTU, propylthiouracil; T3, triiodothyronine; T4, thyroxin.

In test 3, groups of rats were fed diets containing buprofezin at a concentration of 0, 1000 or 5000 ppm or PTU at 200 ppm for 1, 3 or 6 months. Serum concentrations of T3 were only slightly low after 1 month of treatment at 5000 ppm. Serum concentrations of T4 were low in the group at 5000 ppm, with a clear trend for recovery to close-to-normal values as the study progressed (Table 39).

In test 4, groups of rats were given buprofezin at a dose of 0 or 500 mg/kg bw per day or PTU at a dose of 30 mg/kg bw per day by gavage for 15, 30 or 60 consecutive days. After study termination, thyroid weights, serum concentrations of T4 and TP activity were measured and pituitaries were examined histologically.

Relative (and absolute) thyroid weights increased in rats treated with buprofezin or PTU during the study (Table 40). Serum concentrations of T4 were initially decreased in rats treated with buprofezin or PTU but showed a tendency to increase during the study. TP activities were initially increased in rats treated with buprofezin or PTU, then recovered to normal levels at 30 days and increased again thereafter. The pituitaries of rats treated with buprofezin or PTU showed a higher grade of vacuolation than did those of rats in the control group.

To study the direct effects of buprofezin on TP activity, TP activity was measured in vitro by incubation with buprofezin at a concentration of up to approximately 10 µmol/l or similar concentrations of the known TP inhibitors, potassium cyanide (KCN) and PTU. While KCN and PTU completely inhibited TP, full TP activity was observed with buprofezin.

Furthermore, species differences in buprofezin-related changes in serum concentrations of T4 were investigated. In a first test, the dose-related decrease in T4 was shown to correlate with protein-bound iodine (PBI) in rats treated with buprofezin and BPI was also measured as a surrogate for T4 in mice, hamster and guinea-pigs. Repeated doses of buprofezin at 300 or 500 mg/kg bw per day (mice, up to 1000 mg/kg bw) did not significantly change PBI concentrations in these species. In rabbits treated with buprofezin at a dose of 300 or 1000 mg/kg bw per day, a decrease in PBI concentrations was seen from day 2 (Konaka & Nokata, 1989).

The Meeting concluded that buprofezin at a dietary concentration of 1000 ppm (equivalent to a dose of 68.5 mg/kg bw per day in the short-term feeding study by Watanabe, 1986) did not affect serum concentrations of T3 and T4 in rats treated for up to 6 months. Dosing with buprofezin at 500 mg/kg bw per day by gavage for 15–60 days caused increased thyroid weights and decreased serum concentrations of T4 but did not change TP activity significantly. While these effects of bruprofezin are comparable to those of PTU, although with a clearly lower potency, buprofezin did not (in contrast to PTU) show any direct inhibitory effect on TP in vitro.

Table 40. Changes in relative weight of the thyroid, serum concentration of T4 and TP activity (test 4) in rats given buprofezin or PTU by gavage

Test substance	Change (% of control values)								
	Relative thyroid weight			T4 concentration			TP activity		
	Day 15	Day 30	Day 60	Day 15	Day 30	Day 60	Day 15	Day 30	Day 60
Negative control	100	100	100	100	100	100	100	100	100
Buprofezin	129	268	289	11	32	56	161	105	144
PTU	591	1017	1477	5	8	23	227	115	143

From Konaka & Nokata (1989)

PTU, propylthiouracil; T4, thyroxin; TP, thyroid peroxidase.

(b) Induction of ulcers

Groups of 10 male and 10 female F344/DuCrj rats were given buprofezin (purity, 99.5%) as a single dose at 0, 613, 1036, 1751, 2959, or 5000 mg/kg bw by gavage. The study complied with GLP.

At all doses, clinical signs of intoxication were observed and there was high mortality at the two highest doses. On histological examination, induction of duodenal ulcers was observed in males at 1751 mg/kg bw and in females at 2959 mg/kg bw (Ueda, 1985).

(c) Studies on metabolites

(i) BF4, a rat metabolite

The acute oral toxicity of the rat metabolite *tert*-butylhydroxy-buprofezin (BF4) (purity, 99.8%) was investigated in groups of three female Slc:SD rats given a single dose at 300 or 2000 mg/kg bw. The study complied with GLP. The chemical of BF4 is depicted in Figure 2.

No rats died in the group at 300 mg/kg bw, but all rats in the group at 2000 mg/kg bw died within 24 h (Horiuchi, 2004b).

The mutagenic potential of BF4 (purity, 99.8%) was investigated in *S. typhimurium* strains TA100, TA1535, TA98, TA1537 and in *E. coli* WP2 *uvrA* treated at concentrations of 15.4–1250 µg/plate – S9 and 61.7–5000 µg/plate + S9. The study complied with GLP.

No increase in the frequency of revertant colonies was observed at any dose (Inagaki, 2004b).

(ii) BF11, a rat metabolite

The acute oral toxicity of the rat metabolite phenylbiuret (BF11) (purity, 96.9%) was investigated in groups of three female F344/DuCrlCrlj rats given a single dose at 300 or 2000 mg/kg bw. The study complied with GLP. The chemical structure of BF11 is depicted in Figure 2.

No rats died at any dose within 14 days (Nagai, 2008a).

The mutagenic potential of BF11 (purity, 96.9%) was investigated in *S. typhimurium* strains TA100, TA1535, TA98, TA1537 and in *E. coli* WP2 *uvrA* treated at concentrations of 15.4–1250 µg/plate – S9 and 15.4–1250 µg/plate + S9. The study complied with GLP.

No increase in the frequency of revertant colonies was observed at any dose (Inagaki, 2008a).

(iii) BF25, a rat metabolite

The acute oral toxicity of the rat metabolite thiobiuret (BF25) (purity, 92.1%) was investigated in groups of three female F344/DuCrlCrlj rats given a single dose at 300 or 2000 mg/kg bw. The study complied with GLP. The chemical structure of BF25 is depicted in Figure 2.

No rats died in the group receiving the lower dose within 14 days, but all rats in the group receiving the higher dose died within 3 days (Nagai, 2008b).

The mutagenic potential of BF25 (purity, 92.1%) was investigated in *S. typhimurium* strains TA100, TA1535, TA98, TA1537 and in *E. coli* WP2 *uvrA* treated at concentrations of 1.29–5000 µg/plate – S9 and 1.29–1250 µg/plate + S9. The study complied with GLP.

No increase in the frequency of revertant colonies was observed at any dose (Inagaki, 2008b).

(iv) BF26, a plant metabolite

The acute oral toxicity of the plant metabolite BF26 (purity, 95.6%) was investigated in groups of three female Slc:SD rats given a single dose at 50 or 300 mg/kg bw. The study complied with GLP. The chemical structure of BF26 is depicted in Figure 2.

No rats died in the group at 50 mg/kg bw but all rats in the group at 300 mg/kg bw died within 15 min (Horiuchi, 2004a).

The mutagenic potential of BF26 (purity, 95.6%) was investigated in *S. typhimurium* strains TA100, TA1535, TA98, TA1537 and in *E. coli* WP2 *uvrA* treated at concentrations of 61.7–5000 µg/plate ± S9. The study complied with GLP.

No increase in the frequency of revertant colonies was observed at any dose (Inagaki, 2004a).

(v) Structure/activity relationship

The software Derek for Windows® (Lhasa Ltd, UK) was used to identify possible structural alerts in the metabolites BF11, BF26 and 1-*tert*-butyl-3-isopropyl-5-phenyl-2-thiobiuret. No structural alerts were found (Glomski, 2007).

3. Observations in humans

In a medical surveillance report on personnel involved with the synthesis and manufacture of buprofezin between December 1986 and January 1989, no effects attributable to buprofezin were reported (Nokata, 1990).

Comments

Biochemical aspects

Studies with [phenyl-^{14}C]buprofezin showed that the radiolabel was absorbed with a C_{max} at 9 h and was rapidly excreted (> 60% in 24 h and > 80% in 48 h) in male and female rats given doses of 10 and 100 mg/kg bw. In males and females, urinary (22–25%) and faecal (70–74%) cumulative excretion at 10 and 100 mg/kg bw was similar after 4 days. In a study in bile-duct cannulated rats, oral absorption after 24 h was low (40–45%) in males and females; of the administered dose, 30–38% was found in bile, 3–6% in the urine and about 5% in the liver and carcass (not including the gastrointestinal tract). The difference in urinary excretion between bile-duct cannulated and non-cannulated rats suggests that buprofezin excreted in the bile undergoes gastrointestinal re-circulation. The radiolabel was distributed within 2 h to the organs and tissues and after 7 days the highest concentrations were found in erythrocytes, the thyroid and the liver. The total amount of radiolabel recovered in the body accounted for less than 0.7% of the administered dose.

In a 24-week feeding study, no evidence for accumulation was observed. The metabolism of buprofezin was studied in rat liver homogenates and in vivo. Hydroxylation and subsequent methylation of the phenyl ring, oxidation of sulfur with subsequent ring-opening of the thiadiazinane ring and conjugation reactions with sulfate and glucuronic acid were the main metabolic routes. Buprofezin, 4-hydroxybuprofezin (BF2), *tert*-butylhydroxy-buprofezin (BF4), dione metabolite (BF9), buprofezin sulfoxide (BF10), phenylbiuret (BF11), isopropylphenylurea (BF12), 4-hydroxyisopropylphenylurea (BF13), dimethoxy buprofezin (BF20), 4-aminophenol (BF22), 4-hydroxyacetanilide (BF23), thiobiuret (BF25), hydroxy-methoxy-buprofezin (BF27), 2-[3-isopropyl-3-[methylsulfonylmethyl, (phenyl)carbamoyl]ureido]-2-methylpropionic acid (BF28) and dihydroxy buprofezin (C) were identified in the metabolism study in rats.

The results suggested that there are no significant differences between males and females in toxicokinetic parameters and metabolic profiles over a dose range of 10 to 100 mg/kg bw.

Toxicological data

Buprofezin was of low to moderate toxicity when administered orally, with an LD_{50} of 1635–3847 mg/kg bw in rats, LD_{50} > 5000 mg/kg bw in rabbits and LD_{50} > 10 000 mg/kg bw in mice and hamsters. By the dermal, subcutaneous and intraperitoneal routes, the LD_{50}s were > 10 000 mg/kg bw in mice and rats, and the inhalation LC_{50} was > 4.57 mg/L. In rabbits, buprofezin was not irritating to the skin and only very slightly irritating to the eye. In a Magnussen & Kligman maximization test in guinea-pigs, buprofezin gave equivocal results suggesting a very slight potential for delayed contact hypersensitivity, while the results of a local lymph-node assay with buprofezin in mice were negative.

In short-term studies in rats and dogs, the main effects were liver-weight increases accompanied by histological changes; in dogs, behaviour was also affected.

In a 13-week feeding study in rats, the feed intake in males at 200 ppm and above and in females at 5000 ppm was low after 1 or 2 weeks, resulting in lower body weights in the groups at 5000 ppm at study termination. At 200 ppm and above, slight changes in clinical chemistry parameters, including decreased glucose and triglyceride concentrations and increased cholesterol, phospholipid, urea nitrogen and albumin and globulin concentrations were observed. In males and females at 5000 ppm, liver and thyroid weights were increased and spleen weights were decreased. The increases in liver weight were accompanied by hypertrophic and necrotic changes and, in the thyroid, by hypertrophic and hyperplastic changes. The NOAEL was 40 ppm, equal to 3.4 mg/kg bw per day, on the basis of changes in clinical chemistry parameters in rats at 200 ppm.

In a 13-week study in dogs fed capsules containing buprofezin, transiently subdued behaviour was observed 1 h after dosing at 50 mg/kg bw per day and above. This observation was predominantly made in the first few days of treatment, but also at other time-points throughout the study, although with a lower incidence. At 300 mg/kg bw per day, slight ataxia was shown by virtually all dogs 1 h after dosing and persisting for about 5 h. This effect was seen in females only in the first few days of the study, but persisted for 9 weeks in one male. Male and female dogs at the highest dose had significantly lowered body-weight gains, increased liver, kidney and thyroid weights and two- to three-fold increases in the activity of ALP. Increased liver weights were also seen in males and females at 50 mg/kg bw per day. The NOAEL was 10 mg/kg bw per day.

In a 2-year study in dogs given capsules containing buprofezin, which was performed before the 13-week study, no behavioural effects were reported at up to the highest dose of 200 mg/kg bw per day. Increased liver weights were seen in all females receiving buprofezin and in males at 200 mg/kg bw per day. Thyroid weights were high in males and females at 200 mg/kg bw per day. At 20 mg/kg bw per day and above, ALP activity was significantly increased from week 4 onwards and higher incidences of hepatocellular hypertrophy, bile-duct and mammary hyperplasia were found. The NOAEL was 2 mg/kg bw per day.

Since it was not clear whether the observation scheme used in the 2-year study could have detected putative behavioural changes 1 h after treatment, an overall NOAEL for behavioural changes could not be identified. The overall NOAEL for systemic toxicity in the 13-week and the 2-year studies in dogs was 10 mg/kg bw per day on the basis of hepatocellular hypertrophy and bile-duct and mammary hyperplasia at 20 mg/kg bw per day in the 2-year study in dogs.

The long-term toxicity and carcinogenicity of buprofezin has been investigated in mice and rats. The liver was identified as the main target of toxicity.

In the 2-year study in mice, body weights in males and females were slightly (5–10% in females and about 5% in males) but statistically significantly reduced in the group at the highest dose at

5000 ppm from week 6 (males) and week 9 (females) onwards. A very slight trend towards reduced body weight was also observed at 2000 ppm in males and females. At the highest dose of 5000 ppm, males and females had higher platelet counts at study termination and females had transiently lower erythrocyte counts and lower concentrations of haemoglobin. In males and females, liver weights were increased at 2000 ppm and above at 52 weeks; liver weights were statistically significantly increased at study termination only in males at 5000 ppm. Histologically, higher incidences of hepatocellular hypertrophy were seen at 2000 ppm and above. Hyperplastic changes were increased in the livers of males at 5000 ppm and of females at 200 ppm and above, without a clear dose–response relationship in females. In females at 2000 or 5000 ppm, slightly increased incidences of liver adenoma were close to the upper bound of the range for historical controls but without a dose–response relationship. The NOAEL for toxicity was 200 ppm, equal to 17.4 mg/kg bw per day, on the basis of hepatocellular hypertrophy at 2000 ppm. The NOAEL for carcinogenicity was 5000 ppm, equal to 481 mg/kg bw per day, the highest dose tested.

In the 2-year study in rats, terminal body weights were decreased in females at the highest dose of 2000 ppm. Males at 2000 ppm showed lowered activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Males and females at 2000 ppm had elevated liver weights at 26, 52 and 104 weeks. In the first year of the study, rats had higher thyroid weights that were statistically significant only in females. Treatment-related histological changes were restricted to the liver and the thyroid. Since the criteria for histopathological diagnosis had substantially changed since the release of the study report in 1982, the original histology slides for livers and thyroids were re-examined in 1995. At 2000 ppm, males and females had higher incidences for centrilobular hepatocellular and diffuse hypertrophy and females also had more eosinophilic foci than did the controls. A slight and statistically not significant increase in the incidence of liver adenoma was observed at 2000 ppm in females but not in males. Males at 200 ppm and males and females at 2000 ppm showed higher incidences of thyroid F-cell hypertrophy. The NOAEL for toxicity was 20 ppm, equal to 0.9 mg/kg bw per day, on the basis of higher incidences of thyroid F-cell hypertrophy at 200 ppm and the NOAEL for carcinogenicity was 2000 ppm, equal to 89.46 mg/kg bw per day, the highest dose tested.

Buprofezin was not carcinogenic in mice and rats.

Buprofezin was tested for genotoxicity in an adequate range of studies in vitro and for induction of micronucleus formation in vivo. In the submitted studies, there was no evidence for genotoxicity in vitro; however, in a published non-GLP study, micronucleus formation was induced in cultured cells by an aneugenic mechanism, rather than by chromosomal breakage. In assays for micronucleus formation in immature erythrocytes of mouse bone marrow in vivo, conflicting results have been obtained. One study reported statistically significantly increased incidences in two experiments, but the numerical results were very different and were not fully supported by equivocal results from an earlier study in which the administered doses were five times higher. Furthermore, the suggestion of an aneugenic effect in vitro in the published study was not confirmed in vivo.

The Meeting concluded that there was equivocal evidence that buprofezin might be genotoxic.

On the basis of clearly negative results in assays for genotoxicity in vitro and equivocal results in assays for genotoxicity in vivo and the absence of carcinogenicity, the Meeting concluded that buprofezin is unlikely to pose a carcinogenic risk to humans.

The reproductive toxicity of buprofezin has been investigated in two two-generation studies and one one-generation study in rats. In none of the three studies were there any effects on the fertility of males or females or on reproductive performance. In one study, minor increases in liver and kidney weights, without histological correlates, were seen in parental males at 1000 ppm. Pup body weights at birth were not affected by treatment with buprofezin in any of the three studies, but pup body-weight gains were lower at 1000 ppm from postnatal day 4 to postnatal day 21. At postnatal day 21, the body weights of pups at 1000 ppm were 10–18% lower than those of the controls. The NOAEL for

parental toxicity was 100 ppm, equal to 6.46 mg/kg bw per day, on the basis of very slight changes in organ weights. The NOAEL for reproductive effects was 1000 ppm, equal to 66.0 mg/kg bw per day, the highest dose tested. The NOAEL for effects in offspring was 100 ppm, equal to 6.46 mg/kg bw per day, on the basis of reduced pup body-weight gain during lactation at 1000 ppm.

Developmental toxicity with buprofezin had been investigated in rats and rabbits. In rats at the highest dose of 800 mg/kg bw per day, clinical signs of intoxication were observed from day 10 of gestation onwards, dams had lower body-weight gains and lower feed intake from day 7 of gestation onwards and four total litter losses occurred. Additionally, postimplantation losses were increased and fetal body weights were low in this group. At this, the highest dose, there were also more fetuses with a space between body-wall and organs, subcutaneous oedema and retarded ossification. The NOAEL for maternal toxicity and fetal toxicity was 200 mg/kg bw per day on the basis of lower body-weight gains and litter losses in dams and retarded ossification in fetuses at 800 mg/kg bw per day.

In the study of developmental toxicity in artificially inseminated rabbit dams given pooled semen, body-weight gain and feed intake were lowered from the first days of treatment in the group given the highest dose of 250 mg/kg bw per day. One rabbit at 50 mg/kg bw per day aborted and two rabbits at 250 mg/kg bw per day showed total litter losses. Since the frequency of total litter loss was within the range for historical controls, a relationship to treatment is questionable. One fetus from the group at 50 mg/kg bw per day and one fetus from the group at 250 mg/kg bw per day showed unilateral agenesis of one kidney. Because this finding is occasionally observed and might be related to carrier males, its toxicological significance is questionable. Additionally, enlarged aortic arches were observed in one fetus in each of two litters of the group at the highest dose. Although the incidence of this finding was very low it was above the range for historical controls and was thus considered to be treatment-related. The NOAEL for maternal and fetal toxicity was 50 mg/kg bw per day on the basis of lowered body-weight gain and feed intake in dams and increased incidence of enlarged aortic arches in fetuses.

The Meeting concluded that buprofezin was developmentally toxic only at doses that were maternally toxic and did not induce structural changes in fetuses.

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

The Meeting considered that buprofezin is not neurotoxic on the basis of the available data.

In mechanistic studies on thyroid function in rats, buprofezin at a dose of 1000 ppm, equivalent to 68.5 mg/kg bw per day, did not affect serum concentrations of triiodothyronine (T3) and thyroxine (T4). At higher doses, T4 was lowered at the beginning of dosing only and recovered thereafter. At doses of 500 mg/kg bw per day administered by gavage for 15–60 days, thyroid weights increased and concentrations of T4 decreased, but the activity of thyroid peroxidase did not change markedly. The Meeting concluded that the mechanistic studies did not explain the thyroid changes in studies in rats and dogs.

In studies with the rat metabolite BF4, no mortalities were observed in rats given a single oral dose at 300 mg/kg bw, but all rats died at 2000 mg/kg bw. BF4 gave negative results in the Ames test. In studies with the rat metabolite BF11, no mortalities were observed in rats given a single oral dose at 2000 mg/kg bw. BF11 gave negative results in the Ames test. In studies with the rat metabolite BF25, no mortalities were observed in rats given a single oral dose at 300 mg/kg bw, but all rats died at 2000 mg/kg bw. BF25 gave negative results in the Ames test. The plant metabolite BF26 did not induce mortalities in rats given a single oral dose at 50 mg/kg bw, but all rats died at 300 mg/kg bw. BF26 and BF4 gave negative results in the Ames test.

No health effects related to exposure were reported among personnel involved in the synthesis and manufacture of buprofezin.

Toxicological evaluation

The Meeting established an ADI of 0–0.009 mg/kg bw based on a NOAEL of 0.9 mg/kg bw per day in the 2-year study in rats, identified on the basis of increases in the incidence of thyroid F-cell hypertrophy at 8.71 mg/kg bw per day. A safety factor of 100 was applied. The difference between the current ADI and the previous ADI of 0.01 mg/kg bw per day is due to rounding of the figures; both ADIs were based on the same NOAEL from the same study.

The Meeting established an ARfD of 0.5 mg/kg bw based on a NOAEL of 50 mg/kg bw identified on the basis of ataxia at 300 mg/kg bw per day in a 13-week feeding study in dogs. A safety factor of 100 was applied. This ARfD would also be protective against the finding of enlarged aortic arches in rabbit fetuses, although this effect is unlikely to be the result of a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	200 ppm, equal to 17.4 mg/kg bw per day	2000 ppm, equal to 190 mg/kg bw per day
		Carcinogenicity	5000 ppm, equal to 481 mg/kg bw per day[c]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	20 ppm, equal to 0.9 mg/kg bw per day	200 ppm, equal to 8.71 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 89.46 mg/kg bw per day[c]	—
	Two-generation study of reproductive toxicity[a,d]	Reproductive toxicity	1000 ppm, equal to 66.0 mg/kg bw per day[c]	—
		Parental toxicity	100 ppm, equal to 6.46 mg/kg bw per day	1000 ppm, equal to 66.0 mg/kg bw per day
		Offspring toxicity	100 ppm, equal to 6.46 mg/kg bw per day	1000 ppm, equal to 66.0 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	200 mg/kg bw per day	800 mg/kg bw per day
		Embryo and fetal toxicity	200 mg/kg bw per day	800 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
Dog	13-week and 2-year study of toxicity[b, e]	Toxicity	10 mg/kg bw per day	20 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Highest dose tested.

[d] The results for three studies were combined.

[e] The results for two studies were combined.

Estimate of acceptable daily intake for humans

0–0.009 mg/kg bw

Estimate of acute reference dose

0.5 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 buprofezin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 40–45%
Dermal absorption	—
Distribution	Extensive, highest levels in erythrocytes, thyroid, liver
Potential for accumulation	Low, no evidence of accumulation
Rate and extent of excretion	Rapid, > 80% within 48 h, mainly via bile
Metabolism in animals	Extensive, primarily via oxidations, thiadiazinane ring opening and conjugation
Toxicologically significant compounds (animals, plants and environment)	Buprofezin, rat metabolite BF25, plant metabolite BF26
Acute toxicity	
Rat, LD_{50}, oral	1635–3847 mg/kg bw
Rat, LD_{50}, dermal	> 10 000 mg/kg bw
Rat, LC_{50}, inhalation	4.57 mg/l
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization (test method used)	Not a sensitizer (Magnusson & Kligman and local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Feed intake, clinical chemistry (rat) liver weight increase with histological changes (dog)
Lowest relevant oral NOAEL	3.4 mg/kg bw per day (13-week study in rats)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day, highest dose tested (24-day study in rats)
Lowest relevant inhalation NOAEL	No data
Genotoxicity	
	Equivocal evidence of genotoxicity
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Increases in liver weight with histological changes (mouse, rat) and thyroid changes (rat)
Lowest relevant NOAEL	20 ppm, equal to 0.9 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects; reduced body-weight gain in pups during lactation
Lowest relevant reproductive NOAEL	1000 ppm, equal to 66 mg/kg bw per day (rat)

Lowest relevant offspring NOAEL	100 ppm; equal to 6.46 mg/kg bw per day (rat)
Developmental target/critical effect	Enlarged aortic arches (rabbit), retarded ossification (rat)
Lowest relevant developmental NOAEL	50 mg/kg bw per day (rabbit)
Neurotoxicity/delayed neurotoxicity	
	No evidence in conventional studies
Other toxicological studies	
	Rat metabolites BF4 and BF25 and plant metabolite BF26 had moderate acute oral toxicity; the rat metabolite BF11 was of low acute oral toxicity. All metabolites were not genotoxic.
Medical data	
	Medical surveillance of workers in a plant producing buprofezin did not reveal any adverse health effects.

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.009 mg/kg bw	Rat, 2-year study	100
ARfD	0.5 mg/kg bw	Dog, 13-week study	100

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CARBOFURAN (ADDENDUM)

First draft prepared by
Rudolf Pfeil[1] and Les Davies[2]

[1]Toxicology of Pesticides, Federal Institute for Risk Assessment, Berlin, Germany; and
[2]Chemical Review, Australian Pesticides and Veterinary Medicines Authority, Canberra, Australia

Explanation

Carbofuran is the ISO approved common name for 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate, a broad spectrum *N*-methyl carbamate insecticide and nematicide that acts by inhibiting acetylcholinesterase activity in nervous tissues. Carbofuran was previously evaluated by the Joint Meeting in 1976, 1979, 1980, 1982, 1996, and 2002. In 1996, an acceptable daily intake (ADI) of 0–0.002 mg/kg bw was established based on the no-observed-adverse-effect level (NOAEL) for inhibition of erythrocyte acetylcholinesterase at 0.22 mg/kg bw per day in a 4-week dietary study in dogs, and using a safety factor of 100. In 2002, an acute reference dose (ARfD) of 0.009 mg/kg bw was established based on the NOAEL of 0.22 mg/kg bw per day in a 4-week study in dogs, and using a safety factor of 25, as the relevant toxic effects of carbofuran are dependent on the C_{max}.

The present Meeting evaluated newly submitted studies of acute toxicity in rats (adults and pups) and a newly submitted study in human volunteers (conducted in 1976), and re-examined relevant data from short-term studies of toxicity in dogs, which had been considered by previous Meetings. All pivotal studies were certified as complying with good laboratory practice (GLP) or an approved quality assurance programme.

Evaluation for acute reference dose

1. Toxicological studies

1.1 Acute toxicity

(a) Dose range-finding studies in rats aged 11 days

In a dose range-finding study of acute toxicity, groups of three male and three female Crl:CD®(SD) rats were given a single dose of carbofuran (purity, 99%) in corn oil by oral gavage at dose of 0.3, 0.6 or 1.0 mg/kg bw (dose volume, 5 ml/kg bw) on postnatal day 11; there was no concurrent control group. The pups were taken from two lactating dams with reconstituted litters (five males and five females per litter). After dosing, the pups were returned to their dams and examined for clinical signs of toxicity at approximately 15, 30, 60, 90, 120, 240 and 360 min after dosing. At 360 min after dosing, each pup was weighed, examined, terminated by carbon dioxide (CO_2) asphyxiation, and blood was collected via cardiac puncture. Also, the brain was removed, weighed and homogenized for analysis of acetylcholinesterase activity. The assays for acetylcholinesterase enzyme activity were performed by an Ellman method modified for use on an automated clinical chemistry analyser (for details, see Tyl et al., 2005c, below). The study was conducted in accordance with the principles of GLP.

There were no deaths and no pups were found in a moribund condition in any group up to termination at 6 h after dosing. Mean body weights of males and females at 0.3 and 0.6 mg/kg bw were slightly higher at termination than at dosing, while mean body weights at 1.0 mg/kg bw were slightly lower. Mean brain weights were similar across groups at termination for males and females.

Treatment-related clinical signs were first observed at 2 min after dosing. The number of affected rats initially increased with time after dosing as well as with dose. The severity of the signs observed also initially increased with dose and with time after dosing, with peak incidence and severity occurring approximately 10–19 min after dosing, with subsequent reductions in incidence and severity over time to no clinical signs after 42 min after dosing at 0.3 mg/kg bw, after 30 min at 0.6 mg/kg bw, and after 60 min at 1.0 mg/kg bw. The clinical observations were limited to fine tremors initially, then whole-body tremors, and then large head tremors. Over time after dosing, the tremors became intermittent, with large head tremors persisting for the longest time in all three groups receiving carbofuran. Since at age 11 days rat pups are just beginning to open their eyes (most of them have not yet opened their eyes), lacrimation was not observed. As the dams groomed the pups after dosing and while they were experiencing tremors, it was also not possible to observe salivation. The dams and their litters were housed in polycarbonate cages with bedding, and the dams kept their pups in tight piles and groomed them constantly. Therefore, urination and defaecation were also not observed. Once the pups stopped experiencing tremors, they began to nurse and then sleep in the nursing "pile". The slight loss of pup body weight in males and females at 1.0 mg/kg bw between dosing and termination 6 h later is most likely to be because thee pups experienced the longest period of tremors and, therefore, the shortest period of nursing before scheduled termination.

Erythrocyte acetylcholinesterase activity at termination was similar across groups for males and females. Brain acetylcholinesterase activity in males was statistically significantly reduced at 0.6 mg/kg (91% of the value at 0.3 mg/kg bw) and at 1.0 mg/kg (71% of the value at 0.3 mg/kg bw). For females, there were no statistically significantly differences in brain acetylcholinesterase activity between groups (Table 1).

The NOAEL in postnatal day 11 pups was < 0.3 mg/kg bw, based on treatment-related clinical signs (tremors) at a dose of 0.3 mg/kg bw and above. For brain acetylcholinesterase activity, a NOAEL could not be identified since there was no concurrent control group in this study by design (Tyl et al., 2005a).

In a dose range-finding study of acute toxicity, groups of five male and five female Crl:CD®(SD) rats were given a single dose of carbofuran (purity, 98.8%) at 0, 0.03, 0.1 or 0.3 mg/kg bw in corn oil by oral gavage (dose volume, 1 ml/kg bw) on postnatal day 11. Pups from five litters (of four male pups and four female pups per litter) were assigned by consecutive numerical order to the four dosing groups, one pup per sex per litter per group. Clinical observations were recorded before dosing, 1–9 min after dosing and/or 15 min after dosing (before sacrifice for pups from two out of five litters only, due to a deviation from the study protocol). Body weights were recorded once during the period before dosing and on the day of dosing. Samples of whole blood were collected approximately 15 min after dosing, by decapitation. The blood samples were processed for erythrocyte acetylcholinesterase determinations within 15 min and analysis began within 1 h after collection. Immediately after the collection of the blood sample, the brain was excised, weighed and assayed for acetylcholinesterase activity. The assays for acetylcholinesterase enzyme were performed by an Ellman method modified for use on an automated clinical chemistry analyser (for details see Hoberman, 2007c, below). The study was conducted in accordance with the principles of GLP.

There were no deaths. Two of the five males and two of the five females in the group at 0.3 mg/kg bw experienced slight to moderate whole body tremors after dosing, immediately before sacrifice. All other pups survived dosing with no adverse clinical observations. Body weights were comparable between the groups for male and female pups.

No statistically significant reductions in erythrocyte acetylcholinesterase activity occurred for either the male or female pups. Brain acetylcholinesterase activity was significantly reduced in groups at the 0.1 and 0.3 mg/kg bw compared with values for the control group (Table 2).

The NOAEL in pups on postnatal day 11 was 0.03 mg/kg bw on the basis of significant and > 20% inhibition of brain acetylcholinesterase activity at a dose of 0.1 mg/kg bw and above. Treatment-related clinical signs (tremors) were observed at 0.3 mg/kg bw only, the highest dose tested (Hoberman, 2007a).

(b) Time-course studies of acetylcholinesterase inhibition

In a study designed to identify the time of maximum inhibition and the time of complete recovery of acetylcholinesterase activity in erythrocytes and brain, groups of 35 male and 35 female pups at postnatal day 11 and groups of 35 male and 35 female adult (age 60 days) Crl:CD®(SD) rats

Table 1. Relevant findings in rat pups dosed with carbofuran on postnatal day 11

Finding	Dose (mg/kg bw)					
	Males			Females		
	0.3	0.6	1.0	0.3	0.6	1.0
Body weight at dosing (g)	27.34	28.44	25.05	24.70	28.80	25.40
Body weight at termination (g)	29.03	28.67	24.72	25.06	29.32	24.88
Incidence of tremors:						
No. of pups affected	3	2	3	3	2	3
Time-point of observation (min); pup No. 1	8, 19	9	8, 31, 54	15	7, 20	10
Time-point of observation (min); pup No. 2	2, 15	8, 30	13, 14, 19	21	15	8, 13, 35, 60
Time-point of observation (min); pup No. 3	42		2, 7, 10, 14, 16	8		13, 19
Erythrocyte acetylcholinesterase activity (U/l)	3307	3353	3400	2780	3393	3020
Brain acetylcholinesterase activity (U/g)	7.74	7.06**	5.47***	7.01	7.45	6.37

From Tyl et al. (2005a)
** $p < 0.01$; *** $p < 0.001$; compared with the value for the group at 0.3 mg/kg bw.

were given a single dose of carbofuran (purity, 99%) at 0.6 mg/kg bw by gavage in corn oil (dose volume, 5 ml/kg bw). In addition, 10 male and 10 female pups and 10 male and 10 female adults were used as untreated control groups. Clinical observations were recorded before dosing and at approximately 15, 30, 60, 90, 120, 240 and 360 min after dosing (for rats scheduled for sacrifice at each time-point and for rats scheduled for termination at later time-points). At termination, a blood sample (cardiac puncture) and the brain were collected from five male and five female pups and from five male and five female adults each at 15, 30, 60, 90, 120, 240 and 360 min after dosing, and from five male and five female pups and five male and five female adults in the untreated control groups at 0 and 360 min after dosing. The assays for acetylcholinesterase enzyme activity were performed by an Ellman method modified for use on an automated clinical chemistry analyser (for details see Tyl et al., 2005c, below). The study was conducted in accordance with the principles of GLP.

There was no mortality or moribundity in any pup or adult of either sex at 0.6 mg/kg bw at any time-point up to termination 6 h after dosing. In the pups at postnatal day 11, treatment-related clinical signs (whole-body tremors) were observed initially in one (out of thirty-five) males at 2 min after dosing. Tremors were observed in one male 3 min after dosing and in one male and one female at 4 min after dosing, with maximum incidence and severity at 15 min after dosing. Few or no treatment-related clinical observations (tremors) were recorded after 28 min unless the rat was touched or placed in the weighing pan or in the carbon dioxide chamber for termination. Such manipulation triggered tremors that persisted in pups for up to 119 min after dosing. The clinical observations were limited to fine tremors initially, then whole-body tremors, and then large head tremors. With time after dosing, the tremors became intermittent, with large head tremors persisting for the longest period of time. Since at age 11 days rat pups are just beginning to open their eyes (most of them have not yet opened their eyes), lacrimation could not be observed. As the dams groomed the pups after dosing and while they were experiencing tremors, salivation could not be observed. The dams and their litters were housed in polycarbonate cages with bedding, and the dams kept their pups in tight piles and groomed them constantly. Therefore, urination and defaecation could not be observed in the pups.

For the adult rats, the earliest reports of tremors (face) were in one female (out of thirty-five) at 6 min after dosing and one female (out of thirty-five) at 7 min after dosing. The incidences and

Table 2. Relevant findings for rat pups dosed with carbofuran by gavage on postnatal day 11

Finding	Dose (mg/kg bw)							
	Males				Females			
	0	0.03	0.1	0.3	0	0.03	0.1	0.3
Body weight (g)	23.5	24.4	23.9	22.8	21.9	23.4	22.8	23.0
No. of pups with tremors	0	0	0	2[a]	0	0	0	2[a]
Erythrocyte acetylcholinesterase activity (U/l)	1874	1627	1474	1354	2024	1962	1543	1551
Erythrocyte acetylcholinesterase activity (% of values for the control group)	100%	87	79	72	100	97	76	77
Brain acetylcholinesterase activity (U/g)	7.17	6.49	4.76**	2.84**	7.07	6.30	4.62**	3.78**
Brain acetylcholinesterase activity (% of values for the control group)	100	90	66	40	100%	89	65	54

From Hoberman (2007a)

[a] At 15 min after dosing, no clinical observations were recorded for 24 of the 40 pups, owing to a deviation from the study protocol.

** $p < 0.01$.

severities were highest at 15 min, and no tremors were observed after 60 min after dosing. Additional clinical signs included lethargy (in one male at 9 min, one male at 30 min and three males at 15 min after dosing), salivation (in one male at 11 min and in three males at 15 min after dosing), coprophagia (in one female at 15 min, one female at 60 min and two females at 30 min, and in three males at 60 min after dosing), soft faeces (in one male at 30 min), slow respiration (in one male at 60 min), abdominal twitching (in one female at 120 min), and piloerection (in one female at 30 min and in three females at 240 min).

There were no statistically significant effects on erythrocyte acetylcholinesterase activity in pups aged 11 days or adults compared with controls at any time-point (Table 3). However, there was a high variability in erythrocyte acetylcholinesterase activity even in the control group (e.g. means were 3000 ± 466 and 3988 ± 1061 U/l for male pups, and 3192 ± 482 and 4348 ± 1213 U/l for female pups at 0 or 360 min, respectively); this casts doubt on the validity of combining the data for the control group at 0 and 360 min for the statistical analysis. Thus, regarding the quite variable data and the unfavourable assay conditions (see Tyl et al., 2005c, below), the reliability of the assessment of erythrocyte acetylcholinesterase activity in this study is questionable.

Brain acetylcholinesterase activity in male and female pups aged 11 days was statistically significantly inhibited at all time-points examined, with maximum inhibition at 15 min after dosing. Full recovery of brain acetylcholinesterase activity was not achieved in postnatal day 11 pups by 6 h after dosing with a single oral dose of carbofuran at 0.6 mg/kg bw. In adult rats, brain acetylcholinesterase activity was significantly reduced at most time-points, and the time of maximum inhibition was at 15 min after dosing. Recovery of brain acetylcholinesterase activity was achieved in males by 360 min after dosing and in females by 240 min after dosing.

Based on these results, the time of maximum inhibition of brain acetylcholinesterase activity was determined to be 15 min after dosing, in pups and adults. Since there was not full recovery of brain acetylcholinesterase activity in postnatal day 11 pups at 360 min after dosing, the time for full

Table 3. Acetylcholinesterase activity in postnatal day 11 pups and adults given a single dose of carbofuran at 0.6 mg/kg bw

Acetylcholinesterase activity	Time of sacrifice (minutes after dosing)							
	0[a]	15	30	60	90	120	240	360
Pups								
Erythrocytes (U/l):								
Males	3494	2988	3056	4092	3052	3896	3340	4320
Females	3770	3100	3232	4444	3048	3928	3208	5064
Brain (U/g):								
Male	7.33	3.50***	4.02***	4.29***	5.31***	4.62***	5.54***	6.18*
Female	8.38	3.43***	4.45***	4.13***	4.71***	4.99***	6.87*	6.36**
Adults								
Erythrocytes (U/l):								
Male	4208	4332	4456	5124	4228	4732	4796	5164
Female	4760	4088	4432	5272	4908	4540	3856	4536
Brain (U/g):								
Male	12.98	8.04***	8.47***	11.28***	10.74***	11.68**	11.78*	12.00
Female	13.06	8.67***	9.18***	11.38***	11.18***	12.12***	13.04	12.24**

From Tyl et al. (2005b)

[a] The two untreated groups (0 and 360 min sacrifice time) were combined into a single control group.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$;

recovery was selected to be 720 min for a study of the dose–response relationship (see Tyl et al., 2005c, below) (Tyl et al., 2005b).

In a study of acute toxicity designed to identify the time of maximum inhibition and the time of complete recovery of acetylcholinesterase activity in erythrocytes and brain, groups of 60 male and 60 female pups at postnatal day 11and groups of 60 male and 60 female adult Crl:CD®(SD) rats were given a single dose of carbofuran (purity, 98.8%) at 0 or 0.1 mg/kg bw (dose volume, 1 ml/kg bw) in corn oil by gavage. Groups of 10 males and 10 females in the control group and among the rats given carbofuran were assigned to one of six time-points (time between dose administration and sacrifice): 15 min, 30 min, 1 h, 2 h, 4 h and 6 h. The adults and pups were examined for routine clinical observations within the 15 min before dosing and at approximately 15 min, 30 min, 1 h, 2 h, 4 h and 6 h after dosing. Body weights were recorded on the day of dosing. Adults were sacrificed by exsanguination under isoflurane/oxygen anaesthesia during the collection of blood samples at the specified time-points and the pups were sacrificed by decapitation for blood collection. The brains from pups and adults were then excised and weighed. Blood and brain samples were processed and analysed for cholinesterase activity within 2 h of collection. The assays for cholinesterase enzyme activity were performed by an Ellman method modified for use on an automated clinical chemistry analyser (for details, see Hoberman, 2007c, below). The study was conducted in accordance with the principles of GLP.

There were no mortalities. No adverse clinical observations related to the test substance occurred in any adult or pup. There were no treatment-related effects on body or brain weights for adults.

For adults, the time of peak effect on brain acetylcholinesterase activity was determined to be 30 min after dosing for males and females (Table 4). Although erythrocyte acetylcholinesterase activity was increased at 30 min in females, the maximum inhibition of erythrocyte acetylcholinesterase

Table 4. Acetylcholinesterase activity in pups at postnatal day 11 and adults given a single dose of carbofuran at 0.1 mg/kg bw by gavage

Acetylholinesterase activity	Time of sacrifice (minutes after dosing)					
	15	30	60	120	240	360
Pups						
Erythrocyte (% of control):						
Males	70.7	88.4	79.1**	91.0	103.2	123.2
Females	83.9	92.2	85.1	105.2	135.4	102.5
Brain (% of control):						
Males	79.7**	78.7**	73.1**	99.3	101.3	98.5
Females	84.7**	67.7**	67.1**	82.5**	100.4	98.5
Adults						
Erythrocyte (% of control):						
Males	88.0	78.3**	99.7	114.6	81.9**	89.2
Females	83.4**	102.1	89.9	88.9	88.2	114.3
Brain (% of control):						
Males	83.4**	79.1**	83.8*	88.4**	91.4**	95.1
Females	92.1	82.2**	97.3	90.7**	94.5*	97.8

From Hoberman (2007b)
* $p < 0.05$; ** $p < 0.01$.

activity was at 30 min in males, thus supporting the selection of 30 min as the time of peak effect for a subsequent study on dose–response relationship (see Hoberman, 2007c, below).

For pups, the time of peak effect on erythrocyte acetylcholinesterase was between 15 min and 1 h. The time of maximum inhibition of acetylcholinesterase activity in pup brains was at 1 h, although the values for 30 min and 1 h for female pup brain were nearly the same.

The time of peak effect selected for the study on the dose–response relationship was 30 min on the basis of the data for adults and pups presented above. The time of recovery was selected as 4 h for the study on the dose–response relationship, since by this time the pups had recovered and brain acetylcholinesterase activity in adults had reached > 90% of values for the controls (Hoberman, 2007b).

(c) Inhibition of acetylcholinesterase activity in juvenile and adult rats

In a study of acute toxicity designed to determine the dose–response relationship at the times of maximum inhibition and of full recovery of acetylcholinesterase activity in erythrocytes and brain, groups of 10 male and 10 female pups at postnatal day 11 and groups of 10 male and 10 female adult (age 60 days) Crl:CD®(SD) rats were given a single dose of carbofuran (purity, 99%) at 0, 0.3, 0.6 or 1.0 mg/kg bw (dose volume, 5 ml/kg bw) in corn oil by gavage and terminated at approximately 15 min or 720 min after dosing. After dosing, the pups were returned to their dams (18 dams with 5 males and 5 females per litter), and pups and adults were examined for clinical signs of toxicity at 0, 15, 30, 60, 120, 240 and 720 min after dosing. At 15 min or 720 min after dosing, a group of 10 male and 10 female pups and a group of 10 male and 10 female adults per time-point were weighed, examined, terminated by CO_2 asphyxiation, and blood was collected via cardiac puncture. The brain was also removed and weighed. For the assessment of cholinesterase activity, the samples of erythrocytes were washed once and diluted with an equal volume of phosphate buffer at 0.1 mol/l (pH 8.0) containing 1% Triton-X-100, while brains were homogenized in five volumes by weight of phosphate buffer. All samples were stored at -70 °C until analysis. Immediately before analysis, the erythrocyte lysates were further diluted with phosphate buffer, resulting in a final dilution of 1 : 20. The assays for cholinesterase enzyme activity were performed by an Ellman method (Ellman et al., 1961) modified for use on an automated, random-access COBAS MIRA® Plus CC clinical chemistry analyser (Roche Diagnostics, Indianapolis, IN, USA). The assay was conducted at 37 °C with dithiobisnitrobenzoate (DNTB) at 0.25 mmol/l and acetylthiocholine iodide (ACT) at 1 mmol/l, and absorbance was measured at 405 nm. The study was conducted in accordance with the principles of GLP.

All male and female pups and adults survived to the scheduled termination times.

For pups terminated 15 min after dosing, head tremors and tremors not otherwise specified were observed initially in three females 5 min after dosing at 0.3 or 0.6 mg/kg bw. By 6 min after dosing, there was a clear dose–response relationship for incidence of tremors, with maximum incidence and severity until 9 min after dosing. In addition, one male at 0.6 mg/kg bw exhibited gasping and moribundity at 14 min after dosing and was sacrificed on schedule at 15 min after dosing.

For the pups sacrificed 720 min after dosing, tremors were first observed at 5 min after dosing, with a clear dose–response pattern in incidence and severity. The time of maximum response was observed at 15–30 min after dosing in all groups receiving carbofuran. Treatment-related clinical signs (tremors) were present at 0.6 (two rats) and 1.0 mg/kg (eight rats) at 60 min (with nine out of ten rats exhibiting tremors only when touched) and at 1.0 mg/kg (thirteen rats) at 120 min (with twelve out of thirteen rats exhibiting tremors only when touched).

For the adults sacrificed 15 min after dosing, ataxia was observed in one (out of ten) females at 6 min after dosing and in one male at 15 min after dosing, both at 1.0 mg/kg bw, and in one female at 15 min at 0.6 mg/kg bw. Tremors (body, head, facial) began at 6 min at 1.0 mg/kg bw and at 8 min at 0.6 mg/kg bw. The highest incidence and severity of tremors were present at 15 min after dosing, with two males and five females at 1.0 mg/kg bw and one male and two females at 0.6 mg/kg bw.

Piloerection was observed in four males and one female at 0.6 mg/kg bw and in five males and three females at 1.0 mg/kg bw. Lethargy, hindlimb splay, and prone positioning were observed in females at 15 min after dosing at 0.6 mg/kg bw and 1.0 mg/kg bw in a dose-related pattern. There were no treatment-related clinical signs at any time at 0.3 mg/kg bw.

For the adults sacrificed 720 min after dosing, facial tremors were first observed at 7 min in males and females at 1.0 mg/kg bw. Tremors were first observed at 9 min at 0.6 mg/kg bw and at 10 min at 0.3 mg/kg bw. There was a clear dose–response relationship for increased severity of treatment-related clinical observations, with the time of maximum effect being 15–30 min after dosing. Tremors were also observed after 60 min at 0.3, 0.6, and 1.0 mg/kg bw. Other observations included chromodacryorrhoea, lethargy, prone positioning, uneven respiration, slow respiration, coprophagia, salivation, facial fasciculations, jerking head movements, and piloerection at 1.0 mg/kg bw; piloerection, slow respiration, and coprophagia at 0.6 mg/kg bw; and slow respiration, coprophagia, and piloerection at 0.3 mg/kg bw.

Table 5. Clinical signs and acetylcholinesterase activity in pups (postnatal day 11) and adult rats given a single dose of carbofuran by gavage

Finding	Dose (mg/kg bw)							
	Males				Females			
	0	0.3	0.6	1.0	0	0.3	0.6	1.0
Sacrifice at 15 min after dosing								
Pups:								
No. with clinical signs	0	10	10	10	0	10	10	10
Erythrocyte AChE (U/l)	5436	5746	4944	5260	4782	6672	4646	5064
Brain AChE (U/g)	6.62	3.47***	3.13***	2.53***	6.90	3.56***	3.27***	2.42***
Brain AChE (% of control values)	100	52	47	38	100	52	47	35
Adults:								
No. with clinical signs	0	0	5	9	0	0	3	8
Erythrocyte AChE (U/l)	4774	5110	4880	5276	5390	5754	4718	5200
Brain AChE (U/g)	12.46	9.22***	8.44***	7.01***	12.88	9.84***	8.54***	7.16***
Brain AChE (% of control values)	100	74	68	56	100	76	66	56
Sacrifice at 720 min after dosing								
Pups:								
No. with clinical signs	0	10	10	10	0	10	8[a]	10
Erythrocyte AChE (U/l)	4791	4034	6093	4062	4558	4166	5427	4092
Brain AChE (U/g)	7.03	6.49	6.55	6.53	7.17	6.87	7.10	7.20
Brain AChE (% of control values)	100	92	93	93	100	96	99	100
Adults:								
No. with clinical signs	3[b]	5	8	10	0	5	6	10
Erythrocyte AChE (U/l)	5470	5570	5246	6884	5058	5146	5492	5910
Brain AChE (U/g)	13.00	12.29*	12.41	12.18*	13.29	12.74	12.62*	12.55*
Brain AChE (% of control values)	100	95	95	94	100	96	95	94

From Tyl et al. (2005c)
AChE, acetylcholinesterase.
[a] One pup was removed owing to a dosing error.
[b] Piloerection, rough coat.
* $p < 0.05$; ** $p < 0.01$; ** $p < 0.001$.

For pups and adults, there were no statistically significant differences in erythrocyte acetylcholinesterase activity between the control group and any treated group at 15 or 720 min after dosing in males or females. However, in view of the non-optimal assay conditions used in this study (e.g. dilution of samples, incubation time, incubation temperature) which may contribute to significant spontaneous enzyme reactivation (Nostrandt et al., 1993; Wilson et al., 1996; Hunter et al., 1997), the reliability of the assessments of erythrocyte acetylcholinesterase activity in this study is questionable. This conclusion is supported by the results from a recent comparison of two assay methods indicating that the commonly used spectrophotometric method (Ellman et al., 1961) tends to underestimate inhibition of cholinesterase activity in tissues from animals treated with carbamate when compared with the radiometric method (Padilla et al., 2007).

Regarding pup and adult brain acetylcholinesterase activity at the 15-minute time-point, mean values for males and females were statistically significantly reduced at all doses compared with the control group (Table 5). At 720-min, brain acetylcholinesterase activity of the pups was similar in all groups, while brain acetylcholinesterase activity was slightly (≤ 6%) but statistically significantly reduced at 0.3 and 1.0 mg/kg bw in male adults and at 0.6 and 1.0 mg/kg bw in female adults. These differences were not considered to be biologically relevant.

The NOAEL in pups aged 11 days and in adult rats was < 0.3 mg/kg bw on the basis of treatment-related clinical signs and inhibition of brain acetylcholinesterase activity at a dose of 0.3 mg/kg bw and above (Tyl et al., 2005c).

In a study of acute toxicity designed to determine the dose–response relationship at the times of maximum inhibition and of full recovery of acetylcholinesterase activity in erythrocytes and brain, groups of 10 male and 10 female pups at postnatal day 11 and groups of 10 male and 10 female adult Crl:CD®(SD) rats were given a single dose of carbofuran (purity, 98.8%) at 0, 0.03, 0.1 or 0.3 mg/kg bw (dose volume, 1 ml/kg bw) in corn oil by gavage and terminated at approximately 30 min or 240 min after dosing. The pups used for the study were selected from twenty litters; eight pups in each litter were assigned in consecutive numerical order to the four dose groups, one pup per sex per litter per group. The study was conducted in accordance with the principles of GLP.

Adults were examined for clinical observations before and approximately 15 min after dosing, and before sacrifice, at either 30 min or 4 h after dosing. Pups were observed for clinical signs before and at 15 min, 30 min, 1 h and 2 h after dosing. Body weights were recorded on the day of dosing. Motor activity was evaluated 30 min after dosing only for the adult rats designated for sacrifice at 4 h.

Adults were killed by exsanguination under isoflurane/oxygen anaesthesia during collection of blood samples and pups were killed by decapitation via guillotine and blood was collected. Brains were then excised and weighed. For the assessment of cholinesterase activity, blood and brain samples were processed within 10 or 20 min of collection, respectively. Brains were homogenized in 5 ml of 0.1% Tween 80 buffer (pH 8.0), followed by a secondary dilution of 1 : 2 or 1 : 5 for a pup brain or an adult brain, respectively. For erythrocyte samples, the dilution with 0.1% Tween 80 buffer was approximately 1 : 15. All samples were stored on crushed ice during processing and all sample analysis was initiated within approximately 1 h of sampling processing. The assays for cholinesterase enzyme activity were performed by an Ellman method (Ellman et al., 1961) modified for use on an automated clinical chemistry analyser (SpectraMax® 190 microplate reader). The assay was conducted at 26 °C with DNTB at 0.65 mmol/l and ACT at 3.5 mmol/l using a preincubation period of 2 min for erythrocyte and brain homogenates. Absorbance was read at 435 nm at intervals of 15 s, with a run time of 12 min. All samples were routinely analysed in duplicate. Criteria for acceptance of cholinesterase measurements included a duplication rate of 85% for brain and 80% for erythrocyte samples. Samples that did not meet these criteria were noted as “does not replicate” and were re-analysed as soon as possible. Samples that were still coded as “does not replicate” after the second

analysis were re-analysed up to five times, with the final measurement being used in the overall group mean. However, the length of time from collection of blood to the final analysis for those samples requiring additional measurements could not be determined.

There were no mortalities or clinical observations related to dosing with the test substance. There were no effects on body weights, brain weights, or motor activity for either adults or pups.

Adult erythrocyte acetylcholinesterase activity was reduced in a dose-dependent manner at 30 min after dosing and returned to control values 4 h after dosing (Table 6). Pup erythrocyte acetylcholinesterase activity was less sensitive than adults and did not show significant differences from control values at 30 min or 4 h after dosing at any dose. However, additional information from the laboratory performing the assessment revealed that large dilutions, lack of adequate sample mixing, and the time necessary to analyse samples were all likely to have contributed to the high variability and large duplication errors in the study. Therefore, the Meeting considered the data on erythrocyte acetylcholinesterase from this study to be unreliable.

Pup and adult brain acetylcholinesterase activity values were reduced in a dose-dependent manner at 30 min and had essentially recovered by 4 h after dosing, with the exception of male and

Table 6. Acetylcholinesterase activity in pups (postnatal day 11) and adult rats given a single dose of carbofuran by gavage

Finding	Dose (mg/kg bw)							
	Males				Females			
	0	0.03	0.1	0.3	0	0.03	0.1	0.3
Sacrifice at 30 min after dosing								
Pups:								
Erythrocyte AChE (U/l)	826	889	708	617	965	808	609	585
Erythrocyte AChE (% of control values)	100	108	86	75	100	84	63	61
Brain AChE (U/g)	6.00	5.19*	3.88**	2.77**	6.26	5.02**	3.32**	2.67**
Brain AChE (% of control values)	100	87	65	46	100	80	53	43
Adults:								
Erythrocyte AChE (U/l)	1148	1066	815**	707**	1077	906*	854**	664**
Erythrocyte AChE (% of control values)	100	93	71	62	100	84	79	62
Brain AChE (U/g)	14.8	13.1**	10.0**	7.50**	15.1	15.1	12.1**	8.99**
Brain AChE (% of control values)	100	88	68	51	100	100	80	60
Sacrifice at 240 min after dosing								
Pups:								
Erythrocyte AChE (U/l)	518	468	655	532	612	601	589	515
Erythrocyte AChE (% of control values)	100	90	126	103	100	98	96	84
Brain AChE (U/g)	6.23	5.68	5.04**	2.94**	6.37	6.05	4.96**	3.39**
Brain AChE (% of control values)	100	91	81	47	100	95	78	53
Adults:								
Erythrocyte AChE (U/l)	1034	1079	1167	1126	1162	1190	1151	1114
Erythrocyte AChE (% of control values)	100	104	113	109	100	102	99	96
Brain AChE (U/g)	15.7	14.6	13.6**	13.1**	15.9	15.2	14.9	14.5
Brain AChE (% of control values)	100	93	87	84	100	95	94	91

From Hoberman (2007c)
AChE, acetylcholinesterase.
* $p < 0.05$; ** $p < 0.01$.

female pups in the groups at 0.1 and 0.3 mg/kg bw. Statistically significant inhibition of brain acetylcholinesterase activity in the group of pups at 0.03 mg/kg bw recovered to control levels by 4 h.

The NOAEL in pups aged 11 days and in adult rats was 0.03 mg/kg bw on the basis of significant and > 20% inhibition of brain acetylcholinesterase activity at a dose of 0.1 mg/kg bw and above (Hoberman, 2007c).

In a study of acute toxicity designed to compare the sensitivity of inhibition of acetylcholinesterase activity in juvenile and adult rats, groups of 10 male pups (postnatal day 17) and groups of 10 male adult Long-Evans hooded rats were given a single dose of carbofuran (purity, 99%) at 0, 0.1, 0.3, 0.6 or 1.0 mg/kg bw for the pups, or at 0, 0.1, 0.3, 0.5, 0.75 or 1.5 mg/kg bw for the adults, in corn oil by gavage (dose volume, 2 ml/kg bw). The data for the adults were taken from an earlier study (McDaniel et al., 2007), which was carried out under essentially similar conditions. The studies were conducted under an approved intramural research protocol, and data were subjected to a quality assurance review.

At 15 min after dosing, the rats were placed in figure-eight chambers for a 20-min activity session. At approximately 40 min after dosing, the rats were anaesthetized by CO_2 asphyxiation, decapitated, trunk blood was collected and the brain was removed. Erythrocytes and brain tissue were diluted 1 : 3 and homogenized. The measurement of cholinesterase activity was performed using a radiometric cholinesterase assay (Johnson & Russell, 1975). The assay was conducted at 26 °C using an incubation period of 4 min for erythrocytes and 30 s for brain homogenate. After the reaction had been stopped and scintillant had been added, activity was measured using a liquid scintillation counter. Counting efficiency, as determined by an external quench standard, was approximately 62%.

There were no unanticipated deaths or cases of severe toxicity; however, no further details on clinical signs attributed to treatment were reported. For the pups, brain and erythrocyte acetylcholinesterase activity was significantly decreased at all doses of carbofuran, while motor activity was decreased only at doses of 0.3 mg/kg bw and above. Erythrocyte acetylcholinesterase activity was inhibited to a greater degree than brain acetylcholinesterase activity (Table 7).

When compared with adult rats (Table 8), pups aged 17 days showed greater inhibition of acetylcholinesterase activity, even at the lowest dose tested. The effect was slightly greater in erythrocytes than in brain. There was no age-related difference in the inhibition of motor activity by carbofuran at lower doses.

Pearson correlation coefficients of within-subject data were higher for brain acetylcholinesterase and motor activity when compared with erythrocyte acetylcholinesterase activity. This result was in general agreement with the overall conclusion of a comparison of acute neurobehavioral

Table 7. Relevant findings in male rat pups (postnatal day 17) given a single dose of carbofuran by gavage[a]

Finding	Dose (mg/kg bw)				
	0	0.1	0.3	0.6	1.0
Erythrocyte AChE (µmol ACh hydrolysed/min/ml)	0.886	0.446	0.243	0.171	0.153
Erythrocyte AChE (% of control values)	100	50	27	19	17
Brain AChE (µmol ACh hydrolysed/min/g)	5.096	3.686	2.626	2.311	1.812
Brain AChE (% of control values)	100	72	52	45	36
Motor activity (counts/20 min)	80.5	93.7	47.8	28.6	3.7
Motor activity (% of control values)	100	116	59	36	5

From Moser et al. (2007a)
AChE, acetylcholinesterase; ACh, acetylcholine.
[a] Statistical analyses were not performed.

(motor activity) and cholinesterase inhibitory effects conducted for a range of *N*-methyl carbamate pesticides, including carbofuran, in rats (McDaniel et al., 2007).

The NOAEL for pups aged 17 days was < 0.1 mg/kg bw on the basis of > 20% inhibition of brain and erythrocyte acetylcholinesterase activity at a dose of 0.1 mg/kg bw and above. The NOAEL for adult rats was 0.1 mg/kg bw on the basis of > 20% inhibition of brain and erythrocyte acetylcholinesterase activity at a dose of 0.3 mg/kg bw and above (Moser et al., 2007a; McDaniel et al., 2007).

In a study of acute toxicity designed to compare the sensitivity of acetylcholinesterase inhibition in juvenile and adult rats, groups of eight male pups (postnatal day 11) and groups of six male adult Long-Evans hooded rats were given a single dose of carbofuran (purity, 99%) at 0, 0.1, 0.3, 0.6 or 1.0 mg/kg bw (dose volume, 2 ml/kg bw) in acetone/corn oil by gavage. At approximately 40 min after dosing, the rats were anaesthetized by CO_2 asphyxiation, decapitated, trunk blood was collected and the brain was removed. Erythrocyte and brain tissue were diluted 1 : 3 and homogenized. The measurement of cholinesterase activity was performed by a radiometric cholinesterase assay (Johnson & Russell, 1975). The assay was conducted at 26 °C using an incubation period of 2 min for erythrocytes and 30 s for brain homogenate. After the reaction was stopped and scintillant was added, activity was measured using a liquid scintillation counter. Counting efficiency, as determined by an external quench standard, was approximately 62%. The study was conducted under an approved intramural research protocol, and data were subjected to a quality assurance review.

There were no unanticipated deaths or cases of severe toxicity; however, no further details on clinical signs attributed to treatment were reported. As a percent of the respective control groups, inhibition of both brain and erythrocyte acetylcholinesterase activity was greater at all doses tested in the pups than in the adults. In both age groups, erythrocyte acetylcholinesterase activity was inhibited to an equal or greater extent than brain (Table 9).

The NOAEL for pups at postnatal day 11 was less than 0.1 mg/kg bw on the basis of > 20% inhibition of brain and erythrocyte acetylcholinesterase activity at a dose of 0.1 mg/kg bw and above. The NOAEL for adult rats was 0.1 mg/kg bw on the basis of > 20% inhibition of brain and erythrocyte acetylcholinesterase activity at a dose of 0.3 mg/kg bw and above (Moser et al., 2007b).

Using the reliable data on brain acetylcholinesterase from three studies conducted with postnatal day 11 pups (Tyl et al., 2005c; Hoberman, 2007c; Moser et al., 2007b), the estimated oral dose resulting in 10% inhibition of brain acetylcholinesterase activity (benchmark dose at the 10% effect level, BMD_{10}) was 0.04 mg/kg bw, while the lower 95% confidence limit for the BMD_{10} ($BMDL_{10}$) was 0.03 mg/kg bw (US EPA, 2008a and 2008b).

Table 8. Relevant findings in adult rats given a single dose of carbofuran by gavage[a]

Finding	Dose (mg/kg bw)					
	0	0.1	0.3	0.5	0.75	1.5
Erythrocte AChE (µmol ACh hydrolysed/min/ml) [b]	0.428	0.366	0.319	0.301	0.252	0.142
Erythrocyte AChE (% of control values) [b]	100	86	75	70	59	33
Brain AChE (µmol ACh hydrolysed/min/g) [b]	7.158	5.895	4.812	4.544	4.411	2.873
Brain AChE (% of control values) [b]	100	82	67	63	62	40
Motor activity (counts/20 min) [c]	229	179	93	114	85	29
Motor activity (% of control values) [c]	100	78	41	50	37	13

From McDaniel et al., (2007); Moser et al. (2007a)
ACh, acetylcholine; AChE, acetylcholinesterase.
[a] Statistical analyses were not performed.
[b] $n = 5$
[c] $n = 10$

1.2 Short-term studies of toxicity

In a study evaluated by the 1996 and 2002 JMPR, groups of four male and four female beagle dogs were given diets containing carbofuran (purity, 99.6%) at a concentration of 0, 10, 70 or 500 ppm, the highest concentration being reduced to 250 ppm after 6 days owing to toxicity, for 13 weeks. These dietary concentrations resulted in mean intakes of 0, 0.43, 3.1 and 11 mg/kg bw per day for males and females combined. Two additional males and two females given the control diet and the diet containing carbofuran at the highest concentration were kept for a further 4 weeks in order to study the reversibility of the effects.

The dogs were examined daily for deaths and clinical signs. Food consumption was recorded daily and food consumption weekly. Tests of hearing were carried before the start of the study, at the end of dosing and at the end of the recovery period. Ophthalmoscopic examinations and electrocardiography were also carried out, with an additional examination after 6 weeks of dosing. Blood samples (for haematology and clinical chemistry) and urine were taken 7 days before the start of the treatment, at 6 and 13 weeks and after the recovery period. For estimation of plasma and erythrocyte cholinesterase activity, blood samples were taken 30 min after the daily 2 h feeding period at 7 days before the start of the treatment, on test days 1 and 3 and at the end of weeks 1, 2, 6 and 13 of the study (also 11 days later for males); and after the recovery period. The cerebellum was taken at autopsy and immediately assayed for acetylcholinesterase activity. Cholinesterase activity was estimated by an Ellman method (Ellman et al., 1961), modified for use on a Technicon AutoAnalyser II. The assay was conducted at 37 °C. However, no further details of the method were given, and it was not clear whether the experimental conditions were appropriate to minimize spontaneous reactivation of the enzyme. Selected organs were processed for histopathological examination.

One dog at 500 ppm died after 5 days, possibly owing to intussusception of the jejunum. Dogs at all doses showed hyperaemia (of the ear pinnae, abdominal skin and oral mucous membranes) and salivation, these signs being most severe early in the study. The clinical signs at the highest dietary concentration included ataxia, vomiting and tachypnoea. At this concentration, there was loss of body weight and decreased food consumption, which recovered when the concentration of carbofuran in the diet was reduced. No treatment-related differences were seen in ophthalmic, electrocardiographic, haematological or clinical chemical parameters, other than cholinesterase activity.

Table 9. Relevant findings for pups (postnatal day 11) and adult rats given a single dose of carbofuran by gavage[a]

Finding	Dose (mg/kg bw)				
	0	0.1	0.3	0.6	1.0
Pups:					
Erythrocte AChE (µmol ACh hydrolysed/min/ml)	0.871	0.407	0.260	0.139	0.096
Erythrocte AChE (% of control values)	100	47	30	16	11
Brain AChE (µmol ACh hydrolysed/min/g)	3.382	2.028	1.435	0.993	0.760
Brain AChE (% of control values)	100	60	42	29	22
Adults:					
Erythrocte AChE (µmol ACh hydrolysed/min/ml)	0.599	0.523	0.307	0.251	0.155
Erythrocte AChE (% of control values)	100	87	51	42	26
Brain AChE (µmol ACh hydrolysed/min/g)	6.690	5.834	4.786	4.325	3.312
Brain AChE (% of control values)	100	87	72	65	50

From Moser et al. (2007b)
ACh, aceylcholine; AChE, acetylcholinesterase.
[a] Statistical analyses were not performed.

Table 10. Plasma, erythrocyte and brain cholinesterase activity in dogs given diets containing carbofuran for 13 weeks

Cholinesterase activity	Dietary concentration (ppm)							
	Male				Female			
	0	10	70	500/250	0	10	70	500/250
Plasma cholinesterase activity *(µmol-SH/ml)*								
Pre-test, day -7	6.65	7.83	7.13	7.83	6.83	5.74	6.01	7.08
% [a]	100	118	107	118	100	84	88	104
Day 1	5.83	5.47	1.93*	0.93*	5.61	4.45	1.82*	1.22*
% [a]	100	94	33	16	100	79	32	22
Day 3	6.11	6.17	2.63*	3.43*	6.36	4.72	2.52	3.22
% [a]	100	101	43	56	100	74	40	51
Day 7	6.04	5.90	2.84*	1.50*	6.11	4.67	2.41*	1.20*
% [a]	100	98	47	25	100	76	39	20
Day 14	6.61	6.49	3.16*	1.79*	6.94	5.52	2.84*	1.80*
% [a]	100	98	48	27	100	80	41	26
Week 6	7.72	7.19	3.81*	1.61*	8.04	6.38	3.49*	2.06*
% [a]	100	93	49	21	100	79	43	26
Week 13	6.86	6.60	3.38*	1.54*	7.76	5.63	3.16*	1.63*
% [a]	100	96	49	22	100	73	41	21
Erythrocyte acetylcholinesterase (µmol-SH/ml)								
Pre-test (day -7)	2.49	2.50	2.78	2.95	2.85	3.06	2.18	2.19
% [a]	100	100	112	118	100	107	76	77
Day 1	2.34	1.71	0.81*	0.31*	2.64	2.18	0.73*	0.37*
% [a]	100	73	35	13	100	83	28	14
Day 3	2.61	2.07	1.15*	1.20*	3.11	2.52	1.05*	0.80*
% [a]	100	79	44	46	100	81	34	26
Day 7	2.66	1.95	1.07*	0.57*	2.96	2.46	1.03*	0.53*
% [a]	100	73	40	21	100	83	35	18
Day 14	2.68	2.05	1.15*	0.68*	3.12	2.54	1.00*	0.63*
% [a]	100	76	43	25	100	81	32	20
Week 6	3.15	2.35	1.33*	0.67*	3.53	3.12	1.30*	0.62*
% [a]	100	75	42	21	100	88	37	18
Week 13	2.37	1.54	0.81*	0.50*	2.22	2.09	0.92*	0.46*
% [a]	100	65	34	21	100	94	41	21
Brain acetylcholinesterase (µmol-SH/g)								
Week 14	4.97	5.43	5.45	4.70	4.45	4.78	4.41	5.26*
% [a]	100	109	110	95	100	107	99	118

From Bloch et al. (1987a)

[a] Percentage of value for control group.

* $p < 0.05$; compared with control group.

Inhibition of plasma cholinesterase activity was observed at the two higher dietary concentrations, starting from day 1 in males, but only exiguous, insignificant inhibition was observed at the lowest concentration (Table 10). In females, biologically significant inhibition of plasma cholinesterase activity was observed at all dietary concentrations. Inhibition of erythrocyte acetylcholinesterase activity was also seen at all three doses, representing 73%, 35% and 13% of that of concurrent controls at 10, 70 and 500 ppm on day 1 in males, when inhibition was generally maximal. In females at 10, 70 and 500 ppm, the activity on day 1 was 83%, 28% and 14% of that of concurrent controls, respectively. No significant inhibition of brain acetylcholinesterase activity was observed. However, since administration of the test substance was terminated on the day before necropsy, the data on brain acetylcholinesterase activity were not considered to be reliable owing to significant spontaneous reactivation of the enzyme.

As biologically significant depression of erythrocyte acetylcholinesterase activity was observed at the lowest dietary concentration (0.43 mg/kg bw, average intake in males and females), at which clinical signs (salivation, hyperaemia) were also observed, no NOAEL could be identified (Bloch et al., 1987a).

In a subsequent study conducted in order to identify a NOAEL with respect to clinical signs and inhibition of cholinesterase activity, groups of four male beagle dogs were fed diets providing carbofuran (purity, 99.6%) at a concentration of 0 or 5 ppm for 4 weeks. The dogs were examined daily for deaths and clinical signs. Blood samples for measurement of plasma and erythrocyte cholinesterase activity were collected within 30 min after food withdrawal. Cholinesterase activity was estimated by an Ellman method (Ellman et al., 1961), modified for use on a Technicon Auto Analyser II. The assay was conducted at 37 °C. However, no further details of the method were given, and it was not clear whether the experimental conditions were appropriate to minimize spontaneous reactivation

Table 11. Plasma and erythrocyte cholinesterase activity in male dogs fed diets containing carbofuran for 4 weeks

Cholinesterase activity	Dietary concentration (ppm)		
	0		5
Plasma cholinesterase	*Units (µmol-SH/ml)*	% of pre-test value	
Pre-test	6.17	100.0	7.13 (100%) [115.6%][b]
Day 1	5.95	96.4	6.17 (86.5%)[a] [103.7%][b]
Day 3	5.90	95.6	5.95 (83.5%)[a] [100.8%][b]
Day 7	6.06	98.2	6.27 (87.9%)[a] [103.5%][b]
Day 14	5.95	96.4	6.27 (87.9%)[a] [105.4%][b]
Day 28	5.79	93.8	5.95 (83.5%)[a] [102.8%][b]
Erythrocyte acetylcholinesterase (µmol-SH/ml)			
Pre-test	3.14	100.0	2.48* (100%) [79.0%][b]
Day 1	2.99	95.2	2.24* (90.3%)[a] [74.9%][b]
Day 3	2.86	91.1	1.80* (72.6%)[a] [62.9%][b]
Day 7	3.23	102.9	2.37* (95.6%)[a] [73.4%][b]
Day 14	3.27	104.1	2.44* (98.4%)[a] [74.6%][b]
Day 28	3.14	100.0	2.18* (87.9%)[a] [69.4%][b]

From Bloch et al. (1987b)
* $p < 0.05$; compared with control group
[a] (% of pre-test value)
[b] [% of control group value]

of the enzyme. Haematology and clinical chemistry investigations were not performed. Also, gross necropsy and histopathology examinations and measurements of brain acetylcholinesterase activity were not conducted since the dogs were not sacrificed at the end of the test.

Treatment with carbofuran had no effects on clinical signs, mortality, body weight, food consumption, or plasma and erythrocyte cholinesterase activity (Table 11). At the pre-test investigation, erythrocyte acetylcholinesterase activity in dogs to be treated with carbofuran was slightly lower than the value for the control group. Similar differences were seen on days 1, 7, 14 and 28. On day 3, a stronger increase in this difference was noted, but this isolated finding was considered to be of no toxicological significance.

The NOAEL in dogs fed diets containing carbofuran was 5 ppm, equal to 0.22 mg/kg bw per day, the highest dose tested (Bloch et al., 1987b).

2. Observations in humans

In a study to determine the threshold toxicity level in humans, groups of healthy male volunteers (Caucasian; age 23–47 years) received capsules containing carbofuran (purity not specified) as a single oral dose at 0.05, 0.10 or 0.25 mg/kg bw. The number of subjects in the assigned dose groups was two, two and four, respectively, while one male received placebo only. The study was conducted in two parts. The first part was an open study (subjects and investigators knew that carbofuran was ingested) to which subjects were admitted in groups of two. The first group received carbofuran at a dose of 0.05 mg/kg bw, and subsequent groups were introduced until intolerance was demonstrated at a dose of 0.25 mg/kg bw. The second part of the study followed a randomized double-blind design, and evaluated placebo in one subject and the threshold toxic dose of 0.25 mg/kg bw in two subjects. The study was conducted in accordance with the ethical standards current at the time (1976). The nature and purpose of this study were explained to the subjects and their written informed consent was obtained before participation in the programme. The consent procedure and form, as well as the protocol outlining the conduct of this study were approved by the Community Review Committee, Inc., a non-profit corporation organized for the protection of subjects participating in human research.

Subjects were chosen on the basis of a medical history review, physical examination, and laboratory determinations conducted within 14 days before the study. Carbofuran was administered as a single dose immediately after breakfast, after which the subjects remained under observation for 24 h and were monitored for clinical signs and symptoms. The medical examination included tests for heart function (electrocardiogram), vitals signs (blood pressure, temperature, respiration rate), vestibular function, vision (pupil size and accommodation), blood analysis (cholinesterase activity in plasma and erythrocytes) and urine analysis. Plasma and erythrocyte cholinesterase activity were determined using a modification of the Ellman method (Ellman et al., 1961) with propionylthiocholine as substrate. Whole blood was not washed but analysed directly from samples collected from finger tips, and the assay was run at 30 °C for 10 min. Subjects were allowed to smoke during the 24 h sample collection period.

In the two subjects receiving carbofuran at a dose at 0.05 mg/kg bw, no clinical signs were noted. The maximum percentage decreases in cholinesterase activity were 11–22% in erythrocyte and 32–36% in plasma, respectively. Erythrocyte acetylcholinesterase activity recovered within 3 h after administration of carbofuran.

The two subjects subsequently given carbofuran at a dose at 0.1 mg/kg bw presented with mild symptoms including headache (subject No. 3) or lightheadedness (subject No. 4). Subject No. 4 also showed changes in cardiovascular parameters including sinus bradycardia and sinus arrhythmia, and exhibited an abnormal vestibular mechanism before dosing and further deterioration at 3, 5 and 24 h after exposure to carbofuran. Since the time at which these symptoms were reported did not correlate with the time when there was inhibition of acetylcholinesterase activity, the symptoms were considered to be unrelated to treatment. The maximum percentage decreases in cholinesterase activity

were 31–33% in erythrocytes and 35–56% in plasma, respectively. Erythrocyte acetylcholinesterase activity recovered by 3 h after administration of carbofuran.

All subjects at 0.25 mg/kg bw exhibited clinical signs characteristic of acetylcholinesterase inhibition. Individual reactions, however, ranged from one brief episode of mild nausea in subject No. 8, multiple symptoms of mild to moderate severity (diaphoresis, unsteady, drowsy; dry mouth, salivation, abdominal pain) in subject No. 5, multiple symptoms of moderate severity (dizziness, diaphoresis, nervous, weakness, nausea) in subject No. 7, to multiple symptoms of mild to severe intensity (salivation, nervous, dry mouth, abdominal pain; drowsy, weakness, vomiting; diaphoresis, nausea, unsteady) in subject No. 6. Regarding examination of vitals signs, a trend toward lower heart rates after treatment was evident among volunteers at 0.25 mg/kg bw. This trend was most pronounced in subject No. 6, who also displayed the most severe signs and symptoms of toxicity. Also, the two volunteers with the severest symptoms (subjects No. 5 and 6) experienced a marked reduction in body temperature, and subject No. 6 revealed a reduction in respiration rate. In subjects No. 7 and No. 8 at 0.25 mg/kg bw, an effect on pupil size with 2 mm miosis manifested within 2 h after treatment. The maximum percentage decreases in cholinesterase activity were 46–63% in erythrocytes and 33–100% in plasma, respectively. Erythrocyte acetylcholinesterase activity recovered by 6 h after administration.

Plasma cholinesterase activity was depressed in virtually all the subjects, including subject No. 9 who received placebo, and no clear dose–effect relationship could be established for this parameter, although at the highest dose, inhibition was present more consistently. In contrast, inhibition of erythrocyte acetylcholinesterase activity showed a clear dose–response relationship, with maximum percentage decreases of 10% for the subject receiving placebo and up to 46–63% in the subjects given carbofuran at a dose of 0.25 mg/kg bw (Table 12).

Table 12. Relevant findings for men given a single dose of carbofuran as capsules

Finding	Dose (mg/kg bw)								
	0	0.05		0.10		0.25			
Subject No.	9	1	2	3	4	5	6	7	8
Age (years)	28	34	47	29	23	44	47	41	37
Body weight (kg)	75.5	61.3	76.0	71.2	60.0	72.2	77.0	81.6	95.0
Height (cm)	183	165	180	173	169	167	169	164	179
Clinical signs; relationship to treatment	None	None	None	Probably unrelated	Probably unrelated	Related	Related	Related	Related
Headache [a]	—	—	—	Mild (9 h and 45 min)	—	—	—	—	—
Lightheadedness [a]	—	—	—	—	Mild (15 min, 10 h and 45 min)	—	—	—	—
Nausea [a]	—	—	—	—	—	—	Severe (1 h)	Moderate (2 h 30 min)	Mild (30 min)
Diaphoresis [a]	—	—	—	—	—	Mild (1 h)	Severe (1 h)	Moderate (1 h)	—
Dry mouth [a]	—	—	—	—	—	Moderate (1 h)	Mild (3 h)	—	—
Salivation [a]	—	—	—	—	—	Moderate (1 h)	Mild (1 h)	—	—
Abdominal pain [a]	—	—	—	—	—	Moderate (1 h)	Mild (3 h)	—	—
Unsteady [a]	—	—	—	—	—	Mild (3 h)	Severe (1 h)	—	—

Drowsy [a]	—	—	—	—	—	Mild (2 h)	Moderate 45 min)	—	—
Nervous [a]	—	—	—	—	—	—	Mild (1 h)	Moderate (1 h)	—
Weakness [a]	—	—	—	—	—	—	Moderate (1 h)	Moderate (1 h)	—
Vomiting [a]	—	—	—	—	—	—	Moderate (2 h)	—	—
Dizziness [a]	—	—	—	—	—	—	—	Moderate (1 h)	—
ECG findings; time (hours after treatment)	SB (1–4 h)	—	SB (0–24 h)	SA (1 h) SB (0–4)	SA (0–24 h) SB (1–4 h)	SB (3–4 h)	SB (0–24 h)	SA (1 h) SB (2–4 h)	—
Heart-rate depression	—	—	—	—	—	Yes	Yes	Yes	—
Body-temperature depression	—	—	—	—	—	Yes	Yes	—	—
Respiration-rate reduction	—	—	—	—	—	—	Yes	—	—
Miosis	—	—	—	—	—	—	—	Yes	Yes
Vestibular function	AR	Normal	Normal	Normal	AR	Abnormal	Abnormal	AR	AR
Erythrocyte acetylcholinesterase activity (units not given)									
0 h	342	375	359	462	303	322	237	260	251
0 h (%)	100	100	100	100	100	100	100	100	100
0.5 h (%)	+6	-8	+5	-29	-15	-21	-5	+12	-55
1 h (%)	-10	-22	-11	-33	-31	-58	-63	-46	-59
2 h (%)	+3	ND	ND	ND	ND	-46	-49	-39	-52
3 h (%)	-3	+5	+3	-4	-2	-62	-57	-32	-26
6 h (%)	+28	+8	+7	±0	+12	-8	±0	+6	+5
24 h (%)	+40	+12	+5	+1	+5	+24	+27	+51	+44
Plasma cholinesterase activity (units not given)									
0 h	182	143	202	97	168	165	242	106	177
0 h (%)	100	100	100	100	100	100	100	100	100
0.5 h (%)	-42	-27	-20	-56	-35	-20	-15	-40	-47
1 h (%)	-52	-36	-32*	+18	+2	-12	-12	-67	-71
2 h (%)	-51	ND	ND	ND	ND	-14	-18	-100	-81
3 h (%)	-38	-15	-16	+9	+5	-2	-9	-52	-46
6 h (%)	+10	-26	-24	+5	+2	-53	-33	-5	-7
24 h (%)	-9	+2	-3	+22	+14	-7	-10	-28	-32

From Arnold (1976)

AR, abnormal response after treatment; however, evaluation obscured by abnormal responses before treatment; ECG, electrocardiogram; ND, not determined, SA, sinus arrhythmia; SB, sinus bradycardia.

[a] Severity of clinical signs and onset time (hours after treatment)

In conclusion, when given as a single oral dose at 0.05 mg/kg bw, carbofuran inhibited erythrocyte acetylcholinesterase activity by up to 22% in one of two subjects; at 0.1 mg/kg bw, erythrocyte ace-

tylcholinesterase activity was inhibited by up to 31–33% in two out of two subjects; while at 0.25 mg/kg bw, erythrocyte acetylcholinesterase activity was inhibited by up to 46–63% and treatment-related clinical signs were seen in all four subjects. However, owing to the small sample size, the study could not be used for identification of a NOAEL or lowest-observed-effect level (LOAEL) (Arnold, 1976).

Comments

Toxicological data

Carbofuran is highly toxic after a single oral dose; the median lethal dose (LD_{50}) values in rats ranging from 6 to 18 mg/kg bw and in various other species (including mouse, guinea-pig, rabbit, cat and dog) from 3 to 19 mg/kg bw. The clinical signs of toxicity observed were typical of inhibition of acetylcholinesterase activity. In rats, clinical signs were observed from about 5 min after dosing, and mortality generally occurred within 1 h after dosing.

Two studies of the time-course of inhibition of acetylcholinesterase activity were carried out in adult rats and pups aged 11 days (postnatal day 11). In the first study, after a single dose of carbofuran at 0.6 mg/kg bw, the time of maximum incidence and severity of clinical signs and of maximum inhibition of brain acetylcholinesterase activity was at 15 min after dosing for adults and pups. Recovery of brain acetylcholinesterase activity was achieved in adult males and females within 360 or 240 min after dosing, respectively, while the pups had not fully recovered by 360 min after dosing. In the second study, after a single dose of carbofuran at 0.1 mg/kg bw, no clinical signs were observed, and the time of maximum inhibition of brain acetylcholinesterase activity was at 30 min after dosing for adult rats and at 60 min after dosing for pups aged 11 days. Recovery of brain acetylcholinesterase activity was achieved in adults and in the pups within 240 min after dosing.

Two studies of acute toxicity were conducted to compare inhibition of acetylcholinesterase activity in pups aged 11 days (postnatal day 11) and adult rats, and two range-finding studies of acute toxicity were carried out in rats aged 11 days given carbofuran at doses ranging from 0.03 to 1.0 mg/kg bw. In these studies, a spectrophotometric assay for cholinesterase activity was used. While data on erythrocyte acetylcholinesterase were considered to be unreliable because of non-optimal experimental conditions that led to significant spontaneous enzyme reactivation, the data on brain acetylcholinesterase were considered to be suitable for use in risk assessment because the degree of inhibition of acetylcholinesterase activity agreed with that obtained using the more reliable radiometric assay for cholinesterase activity (see below). Clinical signs (tremors) were observed at doses of 0.3 mg/kg bw and above. On the basis of inhibition of acetylcholinesterase activity in brain (pups, 35–47%; adults, 20–32%) at 0.1 mg/kg bw and above, the overall NOAEL for pups and adults was 0.03 mg/kg bw.

In two studies of acute toxicity designed to compare inhibition of acetylcholinesterase activity in pups (postnatal day 11 or postnatal day 17) and adult rats given carbofuran at doses ranging from 0.1 to 1.5 mg/kg bw and using a radiometric assay for cholinesterase activity, the overall NOAEL for pups (both postnatal day 11 and postnatal day 17) was < 0.1 mg/kg bw on the basis of inhibition of acetylcholinesterase activity in brain (28–40%) and erythrocytes (50–53%) at 0.1 mg/kg bw and above. The overall NOAEL for adult rats was 0.1 mg/kg bw on the basis of inhibition of acetylcholinesterase activity in brain (28–33%) and erythrocytes (25–49%) at 0.3 mg/kg bw and above.

Using the data from three studies in rat pups aged 11 days, the BMD_{10} was 0.04 mg/kg bw, while the $BMDL_{10}$ was 0.03 mg/kg bw.

In the latter two studies, inhibition of erythrocyte acetylcholinesterase activity appeared to be a more sensitive end-point than did inhibition of brain acetylcholinesterase activity. In the absence of data on inhibition of acetylcholinesterase activity in peripheral target tissues, the use of data on erythrocyte acetylcholinesterase activity might thus be considered as surrogate for data on the peripheral nervous system. However, given the quantitative dose–response correlation between clinical signs

of cholinergic toxicity and inhibition of brain acetylcholinesterase activity by a range of *N*-methyl carbamates including carbofuran, the Meeting concluded that the current data support the use of inhibition of brain acetylcholinesterase activity rather than the surrogate measure of erythrocyte acetylcholinesterase activity as the end-point for the risk assessment of carbofuran.

In a 13-week dietary study in dogs, which was evaluated by the Joint Meeting in 1996 and 2002 and re-evaluated by the present Meeting, the LOAEL was 10 ppm, equal to 0.43 mg/kg bw per day. A NOAEL was not identified since significant inhibition of erythrocyte acetylcholinesterase activity and clinical signs were seen on the first day of dosing at the lowest dose. The data on brain acetylcholinesterase activity in this study were not reliable owing to significant recovery of acetylcholinesterase activity at the time-point of necropsy. In a supplementary 4-week study in male dogs, which was evaluated by the Joint Meeting in 1996 and re-evaluated by the present Meeting, brain acetylcholinesterase activity was not examined. The NOAEL for clinical signs and inhibition of erythrocyte acetylcholinesterase activity was 5 ppm in the diet, equal to 0.22 mg/kg bw per day, the highest dose tested. However, since a spectrophotometric assay for cholinesterase activity was used in both studies in dogs and it was not clear whether the experimental conditions were appropriate to minimize reactivation of the enzyme, the reliability of the data on erythrocyte acetylcholinesterase activity is questionable. The Meeting noted that, on the basis of the data on acute toxicity, dogs are not expected to be more sensitive than other species.

In a study in human volunteers, which met the ethical standards prevalent at the time when the research was conducted (1976), groups of two to four men received carbofuran as a single oral dose at 0.05, 0.1 or 0.25 mg/kg bw, while one man received placebo only. At 0.05 mg/kg bw, erythrocyte acetylcholinesterase activity was inhibited by 22% in one of two subjects; at 0.1 mg/kg bw, erythrocyte acetylcholinesterase activity was inhibited by 31–33% in both subjects; and erythrocyte acetylcholinesterase activity was inhibited by 46–63% and treatment-related clinical signs were seen in all four subjects at 0.25 mg/kg bw. Owing to the small sample size, the study could not be used for identification of a NOAEL or LOAEL, but provided information that was useful for the interspecies comparison of sensitivity for the risk assessment.

Toxicological evaluation

The Meeting established an ARfD of 0.001 mg/kg bw based on the overall NOAEL of 0.03 mg/kg bw per day identified on the basis of inhibition of brain acetylcholinesterase activity in rat pups aged 11 days and a safety factor of 25. This NOAEL was supported by the $BMDL_{10}$ of 0.03 mg/kg bw extrapolated from data on inhibition of brain acetylcholinesterase activity from three studies in rat pups aged 11 days. A safety factor of 25 was considered to be appropriate because the acute toxic effects of carbofuran are dependent on C_{max} rather than area under the curve of concentration–time (AUC) and data indicated that the sensitivity of humans and laboratory animals (rats, dogs) to inhibition of acetylcholinesterase activity by carbofuran was similar (see general item of the report for JMPR 2008: *Safety factors for acute C_{max}-dependent effects; specific considerations with respect to carbamates such as carbofuran*). Given the apparent higher sensitivity of younger animals, the ARfD was considered to be adequately protective of infants and children since it was based on the NOAEL from a study in pups aged 11 days.

The Meeting noted that this ARfD was lower than the current acceptable daily intake (ADI) of 0–0.002 mg/kg bw. This is plausible in view of the toxicological characteristics of inhibition of acetylcholinesterase activity by carbofuran, which shows very rapid recovery; long-term exposure can thus be likened to a series of acute exposures. The Meeting therefore concluded that the ADI and ARfD for carbofuran should be based on the same NOAEL and revised the ADI to 0–0.001 mg/kg bw based on the overall NOAEL of 0.03 mg/kg bw from the new studies of acute toxicity in rats and using a safety factor of 25.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute study of toxicity (pups aged 11 days and adults) [a, b]	Inhibition of pup brain acetylcholinesterase activity	0.03 mg/kg bw [c]	0.1 mg/kg bw
		Clinical signs	0.1 mg/kg bw	0.3 mg/kg bw

[a] Gavage administration.
[b] Results of several studies combined.
[c] Supported by a $BMDL_{10}$ of 0.03 mg/kg bw, based on inhibition of brain acetylcholinesterase activity in pups aged 11 days (postnatal day 11).

Estimate of acceptable daily intake for humans

0–0.001 mg/kg bw

Estimate of acute reference dose

0.001 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 exposure

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

Summary			
	Value	*Study*	*Safety factor*
ADI	0–0.001 mg/kg bw	Rat, study of acute toxicity	25
ARfD	0.001 mg/kg bw	Rat, study of acute toxicity	25

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CHLORANTRANILIPROLE

First draft prepared by
G. Wolterink[1] and V. Dellarco[2]

[1]Centre for Substances and Integrated Risk Assessment,
National Institute for Public Health and the Environment, Bilthoven, The Netherlands; and
[2]United States Environmental Protection Agency, Office of Pesticide Programs
Health Effects Division, Washington DC, USA

Explanation

Chlorantraniliprole is the International Organization for Standardization (ISO) approved common name for 3-bromo-*N*-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carboxamide). Chlorantraniliprole (CAS No. 500008-45-7) is an insecticide that operates by a highly specific biochemical mode of action. It binds and activates ryanodine receptors, resulting in depletion of intracellular calcium stores and leading to muscle paralysis and death. Comparative studies have demonstrated that differential selectivity of chlorantraniliprole for insect receptors is more than 350-fold that for mammalian receptors.

Chlorantraniliprole is being evaluated for the first time by the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR). The present JMPR review was based on a global assessment of the substance, which was performed in 2007 by 10 countries under the auspices of the Organization for Economic Co-operation and Development (OECD).

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

Evaluation for accetable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

As part of a preliminary study of oral toxicity, groups of at least five male and five female Crl:CD® (SD)IGS BR rats were given chlorantraniliprole (purity, approximately 100%) at a dose of 0, 25, 100 and 1000 mg/kg bw per day by gavage for 14 consecutive days. Blood was collected from three male rats in each of the groups at 25, 100, and 1000 mg/kg bw on test days 14 and 15 immediately before dosing, and then 30 and 60 min, 2, 4, 8, 12, and 24 h after dosing for determination of plasma concentrations of chlorantraniliprole. On day 14, liver tissue of five male and five female rats per group was processed for hepatic biochemical evaluations (beta-oxidation activity, total and specific cytochrome P450 content). Blood was separated into plasma and erythrocytes. In additional male rats assigned to the groups at 25, 100, and 1000 mg/kg bw per day, fat samples were collected for the purpose of assessing potential bioaccumulation of the test substance.

The area under the curve of concentration–time (AUC) for chlorantraniliprole was not proportional to dose, indicating that absorption was decreased at higher doses. Calculated half-lives for chlorantraniliprole in rats in the groups at 25, 100, and 1000 mg/kg bw per day were 3.4, 3.4, and 4.0 h, respectively). Peak plasma concentrations occurred at 0.25, 0.42 and 2.75 h in the groups at 25, 100, and 1000 mg/kg bw per day. The maximum plasma concentrations (up to 0.48 µg/ml at 25 mg/kg bw) were similar at all doses. The concentrations of the test substance in fat were below the limit of quantitation at 24 h after dosing, indicating no significant accumulation of the parent compound.

In females, chlorantraniliprole was a weak inducer of cytochrome P450 isozyme 3A. This enzyme induction was considered to be related to the administration of chlorantraniliprole, but not adverse, and is consistent with a pharmacological response to increased metabolism. Otherwise, chlorantraniliprole did not alter beta-oxidation activity or total and specific cytochrome P450 content (Munley, 2006a).

In a study performed in accordance with OECD guideline 417, a number of kinetic experiments were carried out with Sprague Dawley Crl:CD®(SD)IGS BR rats. All experiments, except a study to determine radioactive residues in the expired air, were performed with a 1 : 1 µCi/µCi mixture of [benzamide carbonyl ^{14}C]chlorantraniliprole (radiochemical purity, 97%) and [pyrazole-carbonyl ^{14}C]chlorantraniliprole (radiochemical purity, 99%), diluted with chlorantraniliprole technical (purity, 96.45%). In all experiments, the rats were dosed by gavage. The study design is presented in Table 1. Statements of adherence to quality assurance (QA) and GLP were included.

Chlorantraniliprole was readily absorbed after oral administration, although absorption was incomplete and dose-related, with T_{max} values of 5–9 h after the lower dose and 11–12 h after the higher dose. At a dose of 10 mg/kg bw, plasma concentrations peaked at 3.0 and 5.4 µg equivalents/g in males and females, respectively. After 24 h, plasma concentrations in males and females were about 1.4 and 3.6 µg equivalents/g. At 200 mg/kg bw, plasma concentrations peaked at 5.1 and 7.1 µg

Table 1. Design of a study of the absorption, distribution and excretion of radiolabelled chlorantraniliprole in rats treated by gavage

Experiment	Dose (mg/kg bw)	Label[a]	No. of rats		Time of sacrifice (h)	Samples
			Male	Female		
Pharmacokinetics	10	Mix[a]	4	4	120	Plasma, erythrocytes[b]
	200	Mix[a]	4	4	120	Plasma, erythrocytes[b]
Volatiles	10	BC[c]	1	1	48	Exhaled volatiles and CO_2, urine, faeces
	10	PC[c]	1	1	48	Exhaled volatiles and CO_2, urine, faeces
Material balance and tissue distribution (terminal)	0	PEG	1	1	168	Urine, faeces, tissues, carcass[d]
	10	Mix[a]	4	4	168	Urine, faeces, tissues, carcass, cage-wash and feed residue
						Metabolite profile in urine and faeces
	200	Mix[a]	4	4	168	Urine, faeces, tissues, carcass, cage-wash and feed residue
						Metabolite profile in urine and faeces
Tissue distribution						
(T_{max})[e]	10	Mix[a]	4	4	5, 9	Urine, faeces, tissues, carcass, cage-wash and feed residue
	200	Mix[a]	4	4	11, 12	Urine, faeces, tissues, carcass, cage-wash and feed residue
(T_{max}/2)[e]	10	Mix[a]	4	4	21, 41	Urine, faeces, tissues, carcass, cage-wash and feed residue
	200	Mix[a]	4	4	52, 64	Urine, faeces, tissues, carcass, cage-wash and feed residue
Biliary elimination	10	Mix[a]	5[f]	5[f]	48	Bile, urine, faeces, GIT, carcass and cage-wash
						Metabolite profile in bile
	200	Mix[a]	4[g]	4[g]	48	Bile, urine, faeces, GIT, carcass and cage-wash

From Himmelstein (2006a)

BC, [benzamide carbonyl-^{14}C]chlorantraniliprole]; GIT, gastrointestinal tract tissue and contents; PC, [pyrazole carbonyl-^{14}C]-chlorantraniliprole; T_{max}, time at maximum plasma concentration (C_{max}); T_{max}/2, time at half of plasma C_{max}.

[a] 'Mix' indicates that each rat was given a single oral dose containing a mixture of [benzamide carbonyl-^{14}C]chlorantraniliprole and [pyrazole carbonyl-^{14}C]chlorantraniliprole in a 1 : 1 ratio (μCi : μCi) at approximately 30 μCi.

[b] Whole blood was collected from the jugular vein at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, and 120 h, for measurement of concentrations of radioactivity in erythrocytes and plasma.

[c] Each rat was dosed with approximately 30 μCi of [benzamide carbonyl-^{14}C]chlorantraniliprole (BC) or [pyrazole carbonyl-^{14}C]chlorantraniliprole (PC) for determination of exhaled volatiles. Exhaled air was sampled 0–24 h and 24 h after dosing.

[d] Control samples were collected for blank analysis. Urine and faeces were collected at intervals of 0–6 h, 6–12 h, 12–24 h after dosing and every 24 h thereafter until termination on day 7. Urine and faeces were analysed for metabolites.

[e] T_{max} and T_{max}/2 were determined experimentally based on pharmacokinetic data. First and second values for time of sacrifice are for male and female rats, respectively. Urine and faeces were collected at intervals of 0–6 h, 6–12 h, and 12–24 h after dosing and every 24 h thereafter until termination at T_{max} or T_{max}/2. At termination, a range of tissues and organs were collected for analysis of content of radioactivity.

[f] Groups of eight male and eight female rats were given [^{14}C]chlorantraniliprole at a dose of 10 mg/kg bw. At this dose, data were reported for five males and five females that had functional bile-duct cannulae throughout the collection, i.e. 48 h. Urine, faeces and bile were collected at intervals of 0–6 h, 6–12 h, and 12–24 h.

[g] [^{14}C]chlorantraniliprole was administered to four rats per sex at the 200 mg/kg bw dose level all of which had functional cannulas. Urine, faeces and bile were collected at intervals of 0-6, 6-12, 12-24h.

equivalents/g in males and females, respectively. In the experiment with bile-duct cannulated rats, total absorption was 73–85% after a dose of 10 mg/kg bw and 12–13% after a dose of 200 mg/kg bw. At the lower dose, 48 h after dosing 18–30% and 49–53% of the absorbed radiolabel was excreted in urine and bile respectively, while 2–6% and 10–20% was found in tissue and faeces respectively. At the higher dose, 48 h after dosing 4% and 5–7% of the absorbed radiolabel was excreted in the urine and bile, respectively, while 3% and 55–71% was found in tissue and faeces, respectively. ^{14}C residues showed extensive distribution in the tissues. In the rats at the lower dose, 0.8% and 3.3% of the administered dose was recovered from the tissues of males and females, respectively, at 168 h after dosing. At this time-point, tissues of males and females at the higher dose contained 0.2% and 0.5%, respectively, of the administered dose. No significant radioactivity was exhaled as ^{14}C-labelled volatiles or $^{14}CO_2$. Concentrations of ^{14}C residues were lower in erythrocytes and tissues than in plasma. The mean plasma elimination half-lives were shorter in males (38–43 h) than in females (78–82 h) rats (Himmelstein, 2006a).

In a kinetic study that complied with OECD guideline 417, male and female Sprague-Dawley Crl:CD®(SD)IGS BR rats were given up to 14 daily doses of [^{14}C]chlorantraniliprole at 10 mg/kg bw per day by gavage. The experiments were performed with a 1 : 1 µCi/µCi mixture of [benzamide carbonyl ^{14}C]-chlorantraniliprole (radiochemical purity, 97%) and [pyrazole-carbonyl ^{14}C]chlorantraniliprole (radiochemical purity, 99%), diluted with chlorantraniliprole technical (purity, 96.45%). Rats were checked daily for clinical signs of toxicity. In three females per group, ^{14}C residues were quantified in whole blood, plasma, erythrocytes, fat, kidney, liver and muscle on days 5, 9, 12, 17, and 27. An evaluation of the distribution of ^{14}C residues in 21 tissues of three males and three females per group was performed on days 15 and 21. Material balance and rate and extent of urine and faecal excretion by male and female rats was quantified until day 21 (seven days after the last dose). Metabolites in urine and faeces (% of accumulating dose), collected for intervals of 24 h after the first, seventh, and last (fourteenth) day of dosing were profiled. Statements of adherence to QA and GLP were included.

More than 98.4% of the administered dose was recovered. Plasma and tissue concentrations indicated that steady-state kinetic behaviour was reached in male rats after 14 days of dosing. In female rats, concentrations of radiolabel in the plasma and tissue were near steady-state at the end of the 14-day dosing period. At day 15, plasma concentrations peaked at 4.6 and 32 µg equivalents/g in males and females, respectively, these concentrations being about two- and sevenfold higher than 24 h after a single dose at 10 mg/kg bw. The concentrations of ^{14}C residues in tissues were higher in females than in males (2.35% vs 0.35% of the administered dose) at 168 h after the last dose. After dosing, the concentration of ^{14}C residues in the selected tissues of female rats declined, with half-lives ranging from 3.9 to 7.7 days. The half-life in plasma ($T_{1/2}$ = 7.2 days) was approximately twofold that determined from plasma collected for up to 5 days after administration of a single dose ($T_{1/2}$ = 3.4 days; see Himmelstein 2006a). A more extensive evaluation of tissue residues in 21 different tissues produced profiles of concentration and percent of dose that were similar to those observed in the single-dose study. Ratios of concentrations in tissue and plasma were less than 1.

Most of the administered dose was excreted in the faeces (males, 72.9%; females, 81.6%). In the urine, 16.7% and 12.1% of the administered dose was excreted by males and females, respectively. The overall pattern of distribution and excretion for multiple dosing (10 mg/kg bw per day × 14 days) generally resided between the pattern observed for administration of a single low dose (10 mg/kg bw) and a single high dose (200 mg/kg bw) (Himmelstein, 2006b).

1.2 Biotransformation

The metabolism of chlorantraniliprole was investigated in two studies in Sprague-Dawley Crl:CD®(SD)IGS BR rats, performed in accordance with OECD guideline 417. The experiments

were performed with a 1 : 1 (μCi : μCi) mixture of [benzamide carbonyl ^{14}C]chlorantraniliprole (radiochemical purity, 97%) and [pyrazole-carbonyl ^{14}C]chlorantraniliprole (radiochemical purity, 99%), diluted with chlorantraniliprole technical (purity, 96.45%). The rats were given a single dose at 10 or 200 mg/kg bw (Himmelstein, 2006a) or daily doses of 10 mg/kg bw by gavage for 14 days (Himmelstein 2006b). Metabolites were identified and quantified by high-performance liquid chromatography (HPLC) and mass spectrometry (MS) or tandem mass spectrometry (MS/MS). Statements of adherence to QA and GLP were provided.

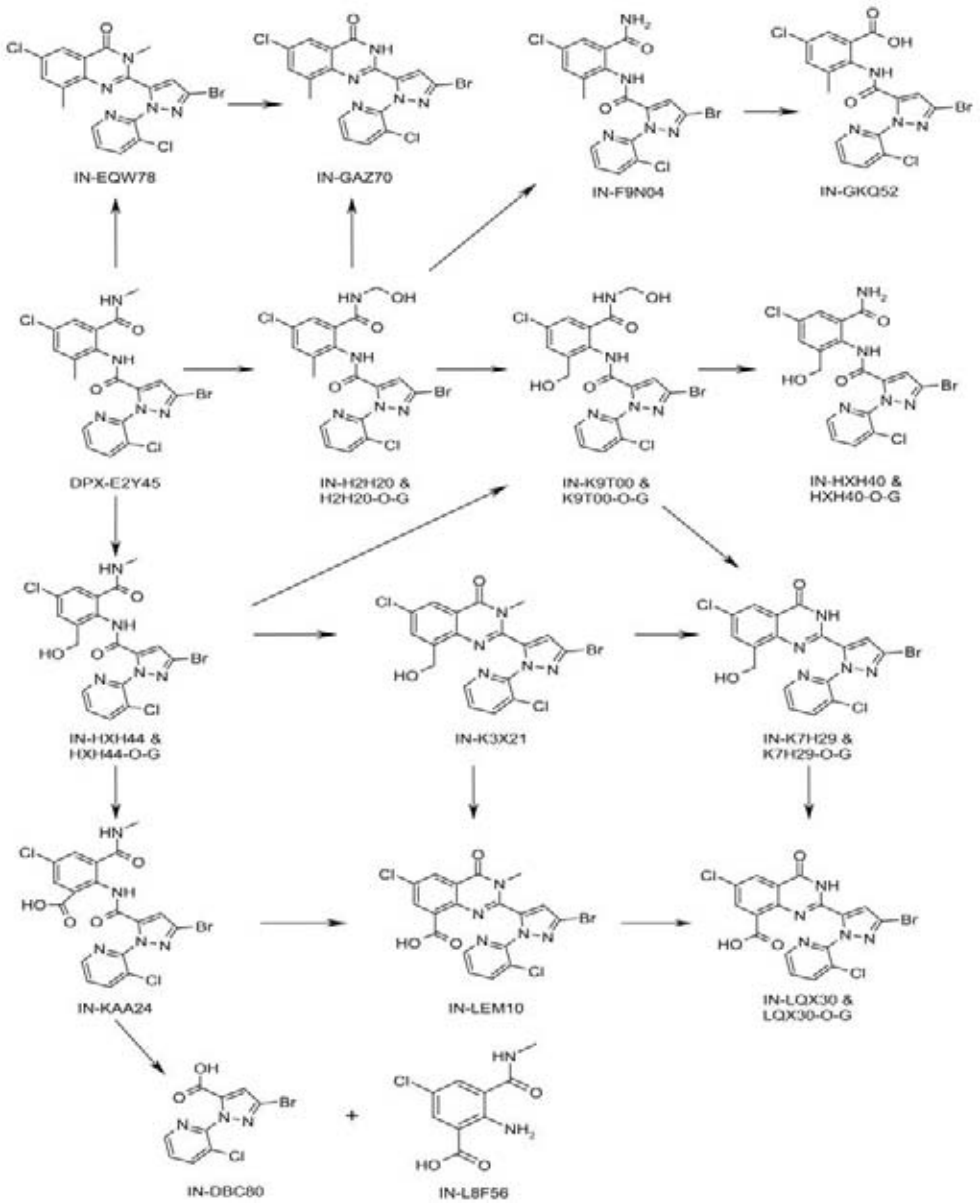
The metabolism of chlorantraniliprole was extensive and characterized by tolyl methyl and *N*-methyl carbon hydroxylation, followed by *N*-demethylation, nitrogen-to-carbon cyclization with loss of a water molecule resulting in the formation of the pyrimidone ring, oxidation of alcohols to carboxylic acids, amide-bridge cleavage, amine hydrolysis, and *O*-glucuronidation. At both doses, a significant difference between the sexes was apparent in the profile of metabolites in the urine and faeces, which indicated greater potential for hydroxylation of the tolyl methyl and *N*-methyl carbon groups in male rats than in female rats. For example, in rats at 10 mg/kg bw, the percentage of the administered dose represented by the di-hydroxylated metabolite IN-K9T00 was greater in males (urine, 7.4%; faeces, 10.4%) than in females (urine, 2.2%; faeces, 4.8%). Concentrations of the methylphenyl mono-hydroxylated metabolite IN-HXH44 were higher in the urine (4.6%) and faeces (7.4%) of males than urine (2.4%) and faeces (3.5%) of females. IN-KAA24, a carboxylic-acid metabolite of IN-HXH44, was a significant metabolite observed in the urine and faeces of males (10.6% combined), but not in females. Percentages of the *N*-methyl carbon hydroxylated metabolite IN-H2H20 were higher in females (urine, 3.4%; faeces, 15.0%) than in males (urine, 0.3%; faeces, 1.4%). At the higher dose, excretion of the parent compound in the urine and faeces (78.9–85.5%) was 12–16-fold that at the lower dose (4.9–7.3%). The profile of metabolites in rats at 200 mg/kg bw was similar to that in rats at 10 mg/kg bw.

The profile of metabolites in the urine and faeces of rats given repeated doses was similar to that observed for rats given single doses. Some minor differences included an apparent increase in the percentages of hydroxylated and polar metabolites such as IN-H2H20, IN-K7H29, and IN-KAA24 after repeated doses. IN-GAZ70 was observed in the faeces of female rats after 7 and 14 days of repeated doses, but not after a single dose. The proposed metabolic pathway is depicted in Figure 1 (Himmelstein, 2006a, 2006b).

As part of a 3-month feeding study in rats, performed in accordance with OECD guideline 408, concentrations of chlorantraniliprole and the two major metabolites, IN-GAZ70 and IN-H2H20 (for structures, see Figure 1) were measured in the plasma. Groups of 10 male and 10 female Crl:CD®(SD) IGS BR rats were given diets containing chlorantraniliprole (purity, 95.9%) at a concentration of 0, 600, 2000, 6000, or 20 000 ppm, equal to 0, 36.9, 120, 359, or 1188 mg/kg bw per day for males and 0, 47.0, 157, 460, or 1526 mg/kg bw per day for females. Concentrations of chlorantraniliprole, IN-GAZ70 and IN-H2H20 were determined by liquid chromatography (LC)/MS in plasma obtained on day 59. Statements of adherence to QA and GLP were provided.

Chlorantraniliprole, IN-GAZ70 and IN-H2H20 were present in the plasma at greater concentrations in female rats (up to 0.83, 112 and 0.54 μg/ml, respectively) than in male rats (up to 0.18, 3.7 and 0.08 μg/ml, respectively) with concentrations of IN-GAZ70 being highest. The plasma concentrations of all three analytes were similar at the three higher dietary concentrations in both sexes (MacKenzie, 2004; Gannon, 2005; Sykes, 2006a).

Figure 1 Proposed metabolic pathway of DPX-E2Y45 in the rat



From Himmelstein (2006a), Himmelstein (2006b)

DPX-E2Y45 (chlorantraniliprole): 3-Bromo-1-(3-chloro-2-pyridinyl)-*N*-[4-chloro-2-methyl-6-[(methylamino)carbonyl] phenyl]-1*H*-pyrazole-5-carboxamide

IN-EQW78: 2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-3, 8-dimethyl-4-(3*H*)-quinazolinone

IN-GAZ70: 2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-8-methyl-4(3*H*)-quinazolinone

IN-F9N04: *N*-[2-(Aminocarbonyl)-4-chloro-6-methylphenyl]-3-bromo-1-(3-chloro-2-pyridinyl)1*H*-pyrazole-5-carboxamide

IN-GKQ52: 2-[[[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]carbonyl]amino]-5-chloro-3-methylbenzoic acid

IN-H2H20: 3-Bromo-*N*-[4-chloro-2-[[(hydroxymethyl)amino]carbonyl]-6-methylphenyl]-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide

IN-H2H20-O-glucuronide: [[2-[[[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl)carbonyl]amino]-5-chloro-3-methylbenzoyl]amino]methyl β-D-glucopyranosiduronic acid

IN-K9T00:	3-Bromo-*N*-[4-chloro-2-(hydroxymethyl)-6-[[(hydroxymethyl)amino)carbonyl] phenyl]-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide
IN-K9T00-O-glucuronide:	[[2-[[[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]carbonyl]amino]-5-chloro-3-(hydroxymethyl)benzoyl]amino]methyl β-D-glucopyranosiduronic acid
IN-HXH40:	*N*-[2-Aminocarbonyl]-4-chloro-6-(hydroxymethyl)phenyl]-3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide
IN-HXH40-O-glucuronide:	[3-(Aminocarbonyl)-2-[[[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]carbonyl]amino]-5-chlorophenyl]methyl β-D-glucopyranosiduronic acid
IN-HXH44:	3-Bromo-*N*-[4-chloro-2-(hydroxymethyl)-6-[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide
IN-HXH44-O-glucuronide:	[2-[[[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]carbonyl]amino]-5-chloro-3-[(methylamino)carbonyl]phenyl]methyl β-D-glucopyranosiduronic acid
IN-K3X21:	2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-8-(hydroxymethyl)-3-methyl-4(3*H*)-quinazolinone
IN-K7H29:	2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-8-(hydroxymethyl)-4(3*H*)-quinazolinone
IN-K7H29-O-glucuronide:	2-[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-1,4-dihydro-4-oxo-8-quinazolinyl]methyl β-D-glucopyranosiduronic acid
IN-KAA24:	2-[[[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]carbonyl]amino]-5-chloro-3-[(methylamino)carbonyl]benzoic acid
IN-LEM10:	2-[5-Bromo-2-(3-chloro-pyridin-2-yl)-2*H* pyrazol-3-yl]-6-chloro-3,4-dihydro-3-methyl-4-oxo-8-quinazolinecarboxylic acid
IN-LQX30:	2-[3-Bromo-1-(3-chloro-2-pyridyl)-1*H*-pyrazol-5-yl]-6-chloro-1,4-dihydro-4-oxo-8-quinazolinecarboxylic acid
IN-LQX30-O- glucuronide:	β-D-Glucopyranuronic acid 1-[2-[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-1,4-dihydro-4-oxo-8-quinazolinecarboxylate
IN-DBC80:	3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxylic acid
IN-L8F56:	2-Amino-5-chloro-3-[(methylamino)carbonyl]benzoic acid

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with chlorantraniliprole are summarized in Table 2. No substance-related clinical signs were observed in studies of oral or dermal toxicity. In the study of toxicity after inhalation, ocular and nasal discharge was observed.

Table 2. Acute toxicity with chlorantraniliprole

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50}/LC_{50} (mg/kg bw; mg/l)	Reference
Rat	Crl:CD®(SD)IGS BR	Female	Oral	Aqueous methylcellulose	96.45	> 5000	Finlay (2004a)[a, b]
Rat	Crl:CD®(SD)IGS BR	Males and females	Dermal	Water	96.45	> 5000	Finlay (2004b) [a, c]
Rat	Crl:CD®(SD)IGS BR	Males and females	Inhalation	Air	96.45	> 5.1	Kegelman (2004) [a, d]

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

[b] Performed according to the up-and-down procedure, OECD guideline 425.

[c] Performed according to OECD guideline 402. Observed clinical signs, i.e. red ocular and red nasal discharge, were attributed to the restraining procedure.

[d] Performed according to OECD guideline 403, nose-only exposure. Mass median aerodynamic diameter (MMAD) was 3.1 μm; geometric standard deviation (GSD) 1.8.

(a) Dermal irritation

In a study of dermal irritation, performed according to OECD guideline 404, three New Zealand White male rabbits were dermally exposed for 4 h to 0.5 g of chlorantraniliprole technical (purity, 96.45%; solid powder) moistened with deionized water. Dermal irritation was scored according to the Draize system at 1, 24, 48 and 72 h after patch removal. Statements of adherence to QA and GLP were included.

No dermal irritation was observed at any point during the study (Finlay, 2004c).

(b) Ocular irritation

In a study of ocular irritation, performed according to OECD guideline 405, 0.1 ml (approximately 72 mg) of chlorantraniliprole technical (purity, 96.45%; solid powder) was instilled into the conjunctival sac of the right eye of one young adult New Zealand White male rabbit. The untreated left eye served as a control. Since no severe irritation or corrosion was observed, two additional rabbits were treated in the same way. Ocular irritation was scored according to the Draize system at 1, 24, 48 and 72 h after instillation. Statements of adherence to QA and GLP were included.

No corneal opacity was noted. Iritis was noted in one eye after 1 h. Conjunctival redness (score, 1) was noted in two out of three eyes at 1 h, persisting in one eye at 24 h and 48 h. Chemosis (score, 1) was noted in two out of three eyes at 1 h. Discharge (scores, 2 and 3) was noted in one eye at 1 h and 24 h. All eyes were free of irritation by 72 h. The Meeting concluded that chloroantraniliprole is not an ocular irritant (Finlay, 2004d).

(c) Dermal sensitization

In a study of dermal sensitization using the local lymph node assay method according to OECD guideline 429, groups of five female CBA/JHsd mice were given chlorantraniliprole technical (purity, 96.45%; solid powder) at a concentration of 0%, 5%, 25%, 50% or 100% (prepared at 1 g/ml in dimethylformanimide) w/v in the vehicle N,N-dimethylformanimide (DMF). The substance used as a positive control was 25% alpha-hexylcinnamaldehyde (HCA). For three consecutive days, the mice in each group were treated with 25 µl of the respective solutions on the dorsal surface of each ear. One group of mice was dosed similarly with the positive control, HCA in 4 : 1 acetone : olive oil (AAO), and one group of mice was dosed similarly with the vehicle used for the positive control only, AAO. On study day 5, the tail vein of each mouse was injected with 20 µCi of [3H]methyl thymidine and the mice were killed 5 h later. The single-cell suspensions of the auricular lymph nodes of each ear were incubated overnight and [3H] activity was counted on day 6. A stimulation index was derived for each experimental group by comparison with the group receiving the vehicle control. A stimulation index of ≥ 3 and/or a statistically significant increased stimulation index were considered to be a positive response. Statements of adherence to QA and GLP were included.

No clinical signs of toxicity or statistically significant differences in body weights and body-weight gains were observed. Stimulation indexes were approximately 1 at all concentrations tested. No statistically significant increases in cell proliferation were observed. The test system was validated by the dermal-sensitization response of the positive control, HCA. In this study, chloroantraniliprole was not a dermal sensitizer (Hoban, 2006).

In a study of dermal sensitization using the Magnusson and Kligman maximization test, according to OECD guideline 406, 20 Dunkin Hartley guinea-pigs were exposed to chlorantraniliprole technical (purity, 96.45%; solid powder). During the preliminary testing phase, the concentrations of the test substance used for the intradermal induction, topical induction and topical challenge,

respectively, were 5% (w/w), 80% (w/w) and 20% (w/w), the highest non-irritating concentration. The vehicle was mineral oil. The control group consisted of 10 guinea-pigs.

The first induction phase involved three paired intradermal injections of 0.1 ml of test substance, 0.1 ml of 50% (v/v) mixture of complete Freund adjuvant in distilled water and 0.1 ml of complete Freund adjuvant with test substance. The guinea-pigs in the control group were treated similarly with vehicle only. The topical induction phase was carried out 1 week later: the guinea-pigs were pre-treated with sodium lauryl sulfate 24 h before topical applications of 0.5 g of chloroantraniliprole on a gauze pad for 48 h under occlusion. In the challenge phase, 21 days after study initiation, the guinea-pigs received topical applications of 0.5 ml of the challenge dose and 0.5 ml of a 33% dilution of the challenge dose for 24 h. At 24 h and 48 h after patch removal, the degree of dermal irritation was scored according to the Magnusson and Kligman grading scale. Data from appropriate historical controls exposed to α-hexylcinnamaldehyde technical (HCA) were used as the positive control. Statements of adherence to QA and GLP were included.

After the intradermal and topical induction phases, very faint to moderate erythema (scores, 0.5–2) was noted at the treatment site in most guinea-pigs receiving chlorantraniliprole and in the control group. At challenge, very faint erythema (score, 0.5) was noted in some guinea-pigs receiving chlorantraniliprole at the highest non-irritating concentration and in some guinea-pigs in the control group. At treatment sites to which the 33% dilution was applied, no dermal reactions were noted. The Meeting concluded that chlorantraniliprole is not a dermal sensitizer under the conditions of the maximization test (Moore, 2004).

2.2 Short-term studies of toxicity

Mice

In a 28-day feeding study, performed in accordance with OECD guideline 407, 10 male and 10 female Crl:CD-1®(ICR)BR mice were fed diets containing chlorantraniliprole (purity, 95.9%) at a concentration of 0, 300, 1000, 3000, or 7000 ppm, equal to 0, 52, 182, 538, and 1443 mg/kg bw per day for males and 0, 64, 206, 658, and 1524 mg/kg bw per day for females (corrected for purity). The mice were observed at least once per day for mortality and clinical signs of toxicity. A detailed clinical examination and body-weight and food-consumption measurements were performed weekly. For five males and five females per group, haematology and clinical chemistry (plasma total protein only) were performed at termination, 4 weeks after initiation of the study. At the same time, gross examinations were performed and selected organs were weighed. Organs and tissues of mice in the control group and in the group at 7000 ppm were examined histologically. On day 13 or 14, five males and five females per group were killed, and peroxisomal β-oxidation and total cytochrome P450 content in liver tissue were determined. Statements of adherence to QA and GLP were included.

At termination, reductions in mean body weight (92% of values for controls) and body-weight gain (43% of values for controls) were observed in males at the highest dose over the 28 days. It was noted that the body-weight gain of the mice in the control group varied greatly during this period, with an initial drop in weight during the first week, followed by a rapid recovery during the following 3 weeks. Weight gain in the groups receiving chlorantraniliprole was constant throughout the treatment period. No dose-dependent effect on body-weight gain was observed in the treatment groups. Although the effects on body weight and body-weight gain in males at the highest dose may have been treatment-related, in isolation, they were considered to be not adverse. The reduction in body-weight gain was accompanied by a reduction in food efficiency (up to 50%). No effect on body weight and food efficiency occurred in females. A slight increase in mean liver weight in 3000 and 7000 ppm females and a mild increase in cytochrome P450 content observed in males and females at 3000 or 7000 ppm were considered to be non-adverse pharmacological responses to metabolism of chlorantraniliprole. Decreased hepatic β-oxidation activity in males at the highest dose (79% of

values for the controls) and females (54% of values for the controls) was also considered to be not adverse, due to the magnitude of change (less than twofold). No histological evidence of organ toxicity was observed. No other parameters were affected.

The NOAEL was 7000 ppm, equal to 1443 mg/kg bw per day, the highest dose tested (Finlay, 2003).

In a 3-month feeding study, performed according to OECD guideline 408, groups of 15 male and 15 female Crl:CD-1®(ICR)BR mice were given diets containing chlorantraniliprole (purity, 95.9%) at a concentration of 0, 200, 700, 2000, or 7000 ppm, equal to 0, 32.6, 115, 345, or 1135 mg/kg bw per day for males and 0, 40.7, 158, 422, or 1539 mg/kg bw per day for females (corrected for purity). The mice were observed at least once per day for mortality and clinical signs of toxicity. A detailed clinical examination and body weight and food consumption measurements were performed weekly. Ophthalmoscopy was performed before treatment and before scheduled termination. At days 92–93 of treatment, blood was collected from all mice for haematology, clinical chemistry and determination of plasma concentrations of chloranatraniliprole and some metabolites. All mice were killed after 3 months. Ten males and ten females per group were examined grossly and selected organs were weighed. An extensive range of organs and tissues of mice from the control group and the group at 7000 ppm was histologically examined. Statements of adherence to QA and GLP were included.

No test substance-related effects were observed on survival, nutritional parameters, haematology, clinical chemistry, clinical or ophthalmological observations. Plasma concentrations of chlorantraniliprole were below the limit of detection in all groups. The plasma concentration of metabolite IN-GAZ70 in females was twofold that in males. In males at 2000 and 7000 ppm, statistically significant reductions in mean body weights (92% and 93% of values for controls, respectively) and mean body-weight gains (67 and 74% of values for controls, respectively) were observed. It was noted that these effects were not dose-dependent, often did not occur on consecutive weeks and were predominantly due to differences in body-weight gain during the last 2 weeks of treatment. Moreover, they were not found in an 18-month study in mice given chlorantraniliprole at similar doses. Therefore the Meeting considered that these effects were not adverse.

The reduction in body-weight gain was accompanied by a reduction in food efficiency. Slight increases in incidences of hyperactivity, hyper-reactivity and convulsions were not dose-dependent, were not confirmed in an 18-month study in mice and were therefore considered to be incidental. No adverse test substance-related effects were observed on organ weights, or any clinical pathology, gross or microscopic pathology endpoints. A slight increase in liver weight in the group at 7000 ppm (9–17%) was considered to be test substance-related but not adverse. The increased liver weights were not associated with any liver histopathology and were attributed to enzyme induction.

The NOAEL was 7000 ppm, equal to 1135 mg/kg bw per day, the highest dose tested (Finlay, 2006a; Gannon, 2006)

Rats

In a preliminary study of oral toxicity, groups of five male and five female Crl:CD®(SD)IGS BR rats were given chlorantraniliprole (purity, about 100%) at a dose of 0, 25, 100 and 1000 mg/kg bw per day by gavage for 14 consecutive days. Data on body weights and clinical observations were collected (at least once) daily. At termination, haematology, blood coagulation, clinical chemistry, and urine analysis were conducted and the rats were examined macroscopically, and selected organs were weighed and examined histopathologically. Bone-marrow smears were examined for the presence of micronuclei to assess potential genetic toxicity; an additional group of rats was given cyclophosphamide to provide a positive control for the presence of genetic toxicity.

There were no test substance-related effects on body weight, clinical observations, haematology, coagulation, clinical chemistry, urine analysis, gross and microscopic pathology, organ weights,

frequency of micronucleated polychromatic erythrocytes or in the ratio of polychromatic erythrocytes to normochromatic erythrocytes (PCEs/NCEs) in bone marrow at any dose tested. There was no microscopic evidence of increased adrenal cortical microvesiculation, which had been observed in some male rats in feeding studies of longer duration (Munley, 2006a).

In a preliminary 28-day feeding study conducted in accordance with OECD guideline 407, groups of five male and five female Crl:CD®(SD)IGS BR rats were given diets containing chlorantraniliprole (purity, 98.6%) at a concentration of 0, 300, 1500, or 8000 ppm, equal to 0, 20.7, 106, and 584 mg/kg bw per day for males and 0, 24, 128, and 675 mg/kg bw per day for females. the rats were observed at least once per day for mortality and morbidity. Clinical signs of toxicity, body weights and food consumption were recorded weekly. Ophthalmological examinations were performed before study start and again before termination. Haematology, clinical chemistry, coagulation tests, bone-marrow smears, urine analysis and measurement of UDP-glucuronyl transferase (UDP-GT) activity in liver tissue were performed at termination 4 weeks after initiation of the study. Also at termination, gross examinations were performed on all rats and selected organs were weighed. Organs and tissues of rats in the control group and in the group at 8000 ppm were examined histologically. In the groups receiving the lowest and intermediate dose, microscopic examinations were conducted on lung, liver, and kidneys. The adrenal glands of male and female rats in the control group and the group at the highest dose were examined microscopically for microvesiculation of the adrenal cortex.

No treatment-related adverse effects were observed. In females, statistically significant increases in relative liver weights (11–14%) and UDP-GT activity (37–51%) were observed in the groups at 1500 and 8000 ppm. Minimal centrilobular hepatocellular hypertrophy was observed in females at 8000 ppm. There was no evidence of hepatic cell damage. The liver effects in females were considered to be treatment-related but not adverse and were attributed to enzyme induction. There was no microscopic evidence of increased adrenal cortical microvesiculation (Donner, 2006a; Sykes, 2006a).

In a 3-month feeding study performed in accordance with OECD guideline 408, groups of 10 male and 10 female Crl:CD®(SD)IGS BR rats were given diets containing chlorantraniliprole (purity, 95.9%) at a concentration of 0, 600, 2000, 6000, or 20 000 ppm, equal to 0, 36.9, 120, 359, or 1188 mg/kg bw per day for males and 0, 47.0, 157, 460, or 1526 mg/kg bw per day for females. The rats were observed at least once per day for clinical signs of toxicity and mortality. Detailed clinical examinations were performed weekly. Body weight and food consumption were measured weekly. Ophthalmological examinations were performed before study start and again before termination. Haematology, clinical chemistry and urine analysis were performed mid-study (days 48–49) and at termination about 3 months after initiation of the study. In plasma obtained at day 59, concentrations of chlorantraniliprole and the two major metabolites, IN-GAZ70 and IN-H2H20 (for structure see Figure 1), were measured by LC/MS. At termination, blood coagulation was tested and bone-marrow smears were examined. Also at termination, gross examinations were performed on all rats and selected organs were weighed. Organs and tissues of rats in the control group and in the group at 20 000 ppm and male hearts and gross lesions observed in males and females at the lowest and intermediate dose were examined histologically. Statements of adherence to QA and GLP were included.

A slight increase in liver weight in the females at 20 000 ppm females (11–17%) and a reduction in bilirubin in females at 2000 ppm and above, not associated with any liver histopathology, were considered to be test substance-related but not adverse and were attributed to enzyme induction. A minimal to mild increase in microvesiculation in the zona fasciculata region of the adrenal cortex in some males at 2000 ppm was considered to be test substance-related but not adverse as the adrenal morphology was within the range for controls, was not associated with cytotoxicity of the adrenal gland and had no impact on adrenal function (assessed in separate studies). No other test substance-related effects were observed.

The NOAEL was 20 000 ppm, equal to 1188 mg/kg bw per day, the highest dose tested (MacKenzie, 2004; Gannon, 2005; Sykes, 2006b).

Dogs

In a 28-day dose range-finding study, groups of two male and two female beagle dogs were given gelatin capsules containing chlorantraniliprole (purity, 97.6%) at a dose of 0, 300 or 1000 mg/kg bw per day (corrected for purity). As part of a subsequent study, groups of four male were given chlorantraniliprole at a dose of 0 or 1000 mg/kg bw per day for 28 days. These additional dogs were randomized into dosing groups on the basis of pre-test testicular volume. All dogs were observed at least twice per day for mortality, morbidity and injury. Detailed clinical examinations and neurobehavioral observations were conducted before the study and then weekly thereafter. Body weight and food consumption were measured weekly. All dogs were examined ophthalmoscopically before the start of the study and again before scheduled termination. Blood and urine were sampled for haematology, clinical chemistry and urine analysis before the test and before termination. In addition, plasma was collected from all dogs on the day before dosing and at 1 h after dosing on test days 1, 2, and 3. Plasma was also collected before dosing and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 18, and 24 h after dosing on day 28. Plasma samples were analysed for concentration of chlorantraniliprole. A sample of liver was collected at necropsy and analysed for hepatic cytochrome P450 (total and isozyme profile: 1A1, 2B1/2, 2E1, 3A2, 4A1). At termination of the study, the dogs were killed and subjected to gross examinations, and selected organs were weighed. An extensive range of tissues was evaluated microscopically.

No treatment-related effects on survival, body weight or nutritional parameters, clinical or neurobehavioral findings, ophthalmology, clinical pathology, or anatomic pathology were observed in dogs exposed to chlorantraniliprole. In the preliminary study with two male and two female dogs per group, testes weights were decreased in a dose-dependent manner (38–49%) at 300 and 1000 mg/kg per day. In these dogs, microscopic changes comprising hypospermatogenesis in the testes, characterized by reduced proportions or total absence of germ cells, accompanied by tubular shrinkage, and Sertoli cell prominence, were observed. However, in the subsequent study in which dogs were randomized into dosing groups on the basis of pre-test testicular volume, no effects on testicular weight were observed. In these dogs, hypospermatogenesis was observed in one out of four dogs in the control group (moderate) and two out of four dogs at 1000 mg/kg per day (minimal). Therefore, given the relatively young age of the dogs, the Meeting concluded that the microscopic testicular observations in the main study were due to sexual immaturity, and not to exposure to chlorantraniliprole.

Oral exposure of male and female dogs to chlorantraniliprole induced hepatic cytochrome P450 enzymes. Although treatment-related, these increases in liver enzymes are not considered to be adverse. Pharmacokinetic parameters (T_{max}, C_{max}, half-life, and AUC) were similar in male and female dogs at 300 mg/kg bw per day. At 1000 mg/kg bw per day, the maximum plasma concentrations and half-lives were similar in males and females; however, the AUC for female dogs was approximately 1.75 fold that for male dogs (Serota, 2003).

In a 28-day feeding study, conducted to investigate potential palatability issues, two groups of two male and two female beagle dogs were given diets containing chlorantraniliprole (purity, 95.9%) at escalating dietary concentrations of 1000 ppm (week 1), 5000 ppm (week 2), and 10 000 ppm (weeks 3–4) for one group and 30 000 ppm (weeks 1–2) and 40 000 ppm (weeks 3–4) ppm for the other group. The mean daily intakes for the groups at 1000, 5000, 10 000, 30 000, and 40 000 ppm were 26, 138, 266, 797, and 1302 mg/kg bw per day in male dogs, and 28, 138, 298, 888, and 1240 mg/kg bw per day in female dogs. A control group of males and two females was fed untreated diet. All dogs were observed at least twice per day for mortality and morbidity. Detailed clinical examinations and neurobehavioral observations were conducted weekly. Body weight was measured

weekly, food consumption was measured daily. At termination of the study, the dogs were killed and subjected to gross examinations.

Palatability of the diet was not affected by inclusion of the test material. Throughout the study, no adverse, test substance-related effects were observed on survival, clinical or neurobehavioral findings, body weight or weight gain, food consumption, or food efficiency. No adverse, test substance-related effects on gross pathology were noted in any dog (Luckett, 2003).

In a 90-day feeding study, performed in accordance with Office of Prevention, Pesticides and Toxic Substances (OPPTS) guideline 870.3150 which resembles OECD guideline 408, groups of four male and four female beagle dogs were given diets containing chlorantraniliprole (purity, 95.9%) at a concentration of 0, 1000, 4000, 10 000, or 40 000 ppm, equal to 0, 32.2, 119, 303 and 1163 mg/kg bw per day for males and 0, 36.5, 133, 318, and 1220 mg/kg bw per day for females (corrected for purity). All dogs were observed at least twice per day for mortality, morbidity and injury. Detailed clinical examinations were conducted twice per day, neurobehavioral observations were conducted weekly. Body weight was measured weekly, food consumption was measured daily. Ophthalmoscopy was performed before start of the study and before termination. Blood and urine samples for haematology, coagulation, clinical chemistry and urine analysis were collected before the test, at week 6 and at week 12. At termination of the study, the dogs were killed and subjected to gross examinations. Selected organs were weighed. An extensive range of organs were examined histologically. Statements of adherence to QA and GLP were included.

No test substance-related effects were observed on mean body-weight gain and nutritional parameters, clinical, neurobehavioral, or ophthalmological signs during the 90 days. No test substance-related effects on clinical pathology, gross or microscopic pathology were observed in dogs exposed to chlorantraniliprole at any dietary concentration. Although not statistically significant, a 20% reduction in body weight and a decrease of approximately 25% in heart weight were reported for females in the group at 10 000 ppm. There were no body-weight reductions in the females at 40 000 ppm and no significant difference in total body-weight gain in females at 10 000 ppm. Furthermore, the 20% reduction in body weight was only experienced in study weeks 11 and 12. Similarly, the decrease in heart weight was not experienced by females at the highest dose and no corresponding microscopic changes were noted in the heart. On the basis of the lack of dose–response for both body weight and heart-weight effects, these effects were not considered to be treatment-related.

Increases in relative liver weights (up to 26%), not dose-dependent, were observed in male dogs in all groups receiving chlorantraniliprole. At 40 000 ppm, the increase (26%) was statistically significant. This finding was not associated with any liver histopathology and was attributed to enzyme induction and was not considered to be adverse.

The NOAEL was 40 000 ppm, equal to 1163 mg/kg bw per day, the highest dose tested (Luckett, 2004).

In a 1-year feeding study, performed in accordance with OECD guideline 452, groups of four male and four female beagle dogs were given diets containing chlorantraniliprole (purity, 96.45%) at a concentration of 0, 1000, 4000, 10 000, or 40 000 ppm, equal to 0, 32, 112, 317, and 1164 mg/kg bw per day for males and 0, 34, 113, 278, and 1233 mg/kg bw per day for females (corrected for purity). All dogs were observed daily for mortality, morbidity and injury. Detailed clinical examinations were conducted, neurobehavioral observations were conducted weekly. Body weight and food consumption were measured weekly. Ophthalmoscopy was performed before start of the study and before termination.

Blood and urine samples for haematology, coagulation, clinical chemistry and urine analysis were collected pre-test, at weeks 13, 26 and 52. At termination of the study, the dogs were killed

and examined grossly. Selected organs were weighed. An extensive range of organs was examined histologically. Statements of adherence to QA and GLP were included.

No test substance-related effects were observed on survival, clinical and neurobehavioral signs, ophthalmology, body weight and nutritional parameters, clinical pathology, or gross or microscopic pathology. Test substance-related increases in absolute and relative liver weights (25–40%) were observed in 40 000 ppm male and female dogs. In the absence of hepatic cell damage the changes in liver weight were considered to be not adverse and attributed to enzyme induction. One male dog in the group at 40 000 ppm demonstrated clinical signs of toxicity, clinical pathology, and anatomic pathology changes consistent with canine juvenile polyarteritis syndrome; these effects were not considered to be test substance-related.

The NOAEL was 40 000 ppm, equal to 1164 mg/kg bw per day, the highest dose tested (Luckett, 2006).

In a 28-day study of dermal administration, performed in accordance with OECD guideline 410, groups of 10 male and 10 female Crl:CD®(SD)IGS BR rats were given chlorantraniliprole (purity, 96.45 %) at a dose of 0, 100, 300, or 1000 mg/kg bw per day (corrected for purity), applied to the shaved, intact dorsal skin under semi-occlusion for 6 h per day for 29 consecutive days. Parameters evaluated included body weight, body-weight gain, food consumption, food efficiency, clinical signs, clinical pathology, organ weights, and gross and microscopic pathology.

All rats were observed daily for mortality, clinical signs and injury. Body weight was recorded twice per week and food consumption was measured weekly. Blood samples were collected on day 29 for haematology, coagulation, and clinical chemistry. At termination of the study, the rats were killed and subjected to gross examinations. Selected organs were weighed. An extensive range of organs of rats in the control group and rats in the group at 1000 mg/kg bw per day, and organs with gross lesions from rats in all groups were examined histologically. In addition, adrenal glands from all groups of males were evaluated histologically. Statements of adherence to QA and GLP were included.

No test substance-related effects were observed on survival, clinical observations or food consumption. No adverse test substance-related effects were observed on organ weights, any clinical pathology, gross or microscopic pathology end-point. Treatment-related reductions in mean body weight (6% and 5% in males and females, respectively) and body-weight gain (22% and 19% in males and females, respectively) and food efficiency were observed over the 28 days in males and females at the highest dose, but these effects were not considered to be adverse. Minimal increases in adrenal microvesiculation in some males at all doses were considered to be test substance-related, but not adverse, as adrenal morphology was within the normal range, was not accompanied by microscopic evidence of toxicity and had no impact on adrenal function (assessed in a separate study).

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Finlay, 2006b).

In a 28-day study of dermal administration, 10 male Crl:CD®(SD)IGS BR rats were given chlorantraniliprole (purity, 96.45%) at a dose of 1000 mg/kg bw per day applied to the shaved, intact dorsal skin for 6 h per day for 29 daily (consecutive) applications. A control group of 10 male rats was treated with deionized water in a similar manner. A control group of 10 male rats underwent no shaving, application, or wrapping. Rats were observed daily for clinical signs after removal of the test substance. Body weight and food consumption were measured weekly. On the day following the last dermal treatment, each rat received an intravenous injection of adrenal corticotropic hormone (ACTH) and blood was collected for determination of corticosterone concentration. After blood collection, the rats were killed and the adrenal glands were examined microscopically. Statements of adherence to QA and GLP were included.

Although a significant reduction in body-weight gain after the first week of dosing (a decrease of 75% compared with the control group receiving deionized water) was observed, absolute body

weights of the treated rats was never less than 95% of that of the control group receiving deionized water. Similar effects were noted on food efficiency. There was a greater incidence of increased adrenal cortical microvesiculation in rats given chlorantraniliprole and stimulated with ACTH when compared to both groups of in-study control rats (non-wrapped control group, 0 out of 10; control group receiving deionized water, 1 out of 10; group receiving chlorantraniliprole, 4 out of 10). There were no effects on ACTH-stimulated serum corticosterone concentrations in rats treated dermally with chlorantraniliprole compared with the concurrent control group receiving deionized water.

The study indicated that dermal exposure to chloranatraniliprole does not affect adrenal corticosterone function (synthesis and release) at a dose that results in an increased degree of adrenal cortical microvesiculation (Finlay, 2006c).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In an 18-month feeding study of carcinogenicity, performed in accordance with OECD guideline 451, groups of 70 male and 70 female Crl:CD-1®(ICR)BR mice were given diets containing chlorantraniliprole (purity, 96.45%) at a dose of 0, 20, 70, 200, 1200, or 7000 ppm, equal to 0, 2.60, 9.20, 26.1, 158 and 935 mg/kg bw per day in males and 0, 3.34, 11.6, 32.9, 196 and 1155 mg/kg bw per day in females (corrected for purity). Mice were checked daily for clinical signs, and a detailed clinical examination was performed weekly. Body weights and food consumption were recorded weekly for the first 13 weeks and every other week thereafter. Ten male and ten female mice per group were tested by functional operational battery (FOB) before the start of the treatment and on days 45, 90 and 180 (approximately). Ophthalmological examinations were performed before treatment and before termination. Leukocyte relative differential counts were performed on blood smears of mice killed in extremis and on mice in the control group and at the highest dose at 18 months. At termination, all mice were examined grossly. Selected organs were weighed. An extensive range of tissues from rats in the control group and at the highest dose and all decedent mice were examined microscopically. Gross observations, observed at necropsy and male livers (i.e. suspected target organ) were examined microscopically for all rats. Statements of adherence to QA and GLP were included.

Absolute and relative liver weights showed a dose-related increase at 1200 (6–11%) and 7000 ppm (15–19%). Statistically significant increased hepatocellular hypertrophy was observed in males at 1200 and 7000 ppm. These effects are consistent with pharmacological enzyme induction and in isolation they are not considered to be adverse. In males at 7000 ppm, an increased incidence (7.1%; historical control range, 0–1.9%) of eosinophilic foci of cellular alteration (slightly nodular, focal, cluster of enlarged "eosinophilic" hepatocytes within the hepatic parenchyma) was observed. No information on the chemical-specific mechanism of action was available to evaluate the relevance of liver foci to exposure of humans. However, since these eosinophilic foci may potentially be preneoplastic lesions and are likely to be test substance-related, they are considered to be an adverse effect. The incidence of bronchioloalveolar adenoma was slightly increased (not statistically significant) in males at 7000 ppm; however, the combined incidence of bronchioloalveolar adenoma and carcinoma was similar in males at 7000 ppm and in the controls. Although statistically significant increases of malignant lymphoma metastasis were observed in females at 7000 ppm, the incidence of primary malignant lymphoma was not statistically significantly increased. No dose–response relationship was observed in the incidence of malignant lymphoma in mice at the intermediate dose, although it should be noted that haemolymphatic tissue was evaluated in only about half the mice at the intermediate dose. Historical data on the incidence of lymphoma were not provided. No increase in the incidence of lymphoma was observed in males at any dose. The Meeting concluded that there was no test substance-related increase in tumour incidence.

The NOAEL was 1200 ppm, equal to 158 mg/kg bw per day, on the basis of the presence of eosinophilic foci accompanied by hepatocellular hypertrophy and increased liver weight in males at 7000 ppm (Finlay, 2006d)

Rats

In a 2-year combined study of toxicity and carcinogenicity, performed in accordance with OECD guideline 453, groups of 70 male and 70 female Crl:CD®(SD)IGS BR rats were given diets containing chlorantraniliprole (purity, 96.45%) at a concentration of 0, 200, 1000, 4000, or 20 000 ppm, equal to 0, 7.71, 39.0, 156, and 805 mg/kg bw per day in males and 0, 10.9, 51.0, 212, and 1076 mg/kg bw per day in females (corrected for purity), for approximately 23 months. An interim sacrifice of 10 male and 10 female rats per group was conducted after 1 year. The rats were observed daily for mortality, morbidity and clinical signs. Detailed clinical examinations were conducted weekly. Body weight and food consumption were measured weekly for the first 13 weeks, and once every 2 weeks thereafter. Ophthalmoscopy was performed before start of the study, at day 366 and at day 660 before termination. Blood and urine samples for haematology, clinical chemistry and urine analysis were collected from 10 males and 10 females per group pre-test, and after about 3, 6 and 12 months. For these same 10 males and 10 females per group, coagulation measurements in blood and corticosterone measurements in urine were performed at 1 year and they were killed for interim pathological examinations. From all other surviving rats in the control group and at the highest dose, blood smears collected at 23 months were evaluated for leukocyte differential count.

All interim-kill rats and rats surviving until 23 months were subjected to gross pathology and selected organs were weighed. A wide range of tissues collected from rats in the control group and at the highest dose were evaluated microscopically. In groups at the intermediate and lowest dose, microscopic examinations were also conducted on the adrenal glands of males from both scheduled sacrifices and on thyroid glands of females at the terminal sacrifice. Gross lesions from all rats were examined microscopically. All rats sacrificed in extremis, found dead, or accidentally killed were examined grossly and all collected tissues were evaluated microscopically. A portion of adrenal gland was evaluated by electron microscopy in four male rats in the control group and four male rats in the group at 20 000 ppm sacrificed after 1 year. Statements of adherence to QA and GLP were included.

Rats were sacrificed before 2 years due to declining survival in most groups in both sexes, including the controls. The deaths were not due to exposure to chlorantraniliprole as there was no statistically significant test substance-related effect on mortality. Survival on test day 693 (before scheduled sacrifice) in males at 0, 200, 1000, 4000, and 20 000 ppm was 37%, 42%, 45%, 37% and 47%, respectively. Survival on test day 686 (before scheduled sacrifice) in females at 0, 200, 1000, 4000, and 20 000 ppm was 34%, 45%, 33%, 40%, and 35%, respectively. No treatment-related effects on clinical signs, body weight, body-weight gain, food consumption, food efficiency, ophthalmology, haematology, coagulation, clinical chemistry, urine analysis, and urine corticosterone evaluations or on leukocyte differential counts were observed.

An increase in relative liver weights was observed in female rats at 4000 ppm (14%) and 20 000 ppm (24%) (only at interim sacrifice at 1 year), but was not associated with any findings indicative of liver toxicity. Therefore, these weight changes were considered non-adverse and consistent with a pharmacological response to metabolism. No gross pathology findings were attributed to exposure to chlorantraniliprole. Increased adrenal cortical microvesiculation due to lipid was present in the zona fasciculata region of the adrenal gland of some male rats at all doses in the 1-year study and in the main study. This finding was considered to be related to administration of chlorantraniliprole, but was not considered to be adverse since microscopic and electron microscopic examination showed that the adrenal morphology was generally in the range of what was observed in rats in the control group, and the finding was not associated with any indication of cytotoxicity or other evidence of structural or functional impairment (corticosterone concentrations in urine were normal)

of the adrenal gland. No other treatment-related microscopic changes were observed in males or females. At the doses tested, chlorantraniliprole was not carcinogenic in male or female rats.

The NOAEL was 20 000 ppm, equal to 805 mg/kg bw per day, the highest dietary concentration tested (MacKenzie, 2006).

2.4 Reproductive toxicity

(a) Multigeneration study

Rats

In a 2-generation study of reproductive toxicity, performed in accordance with OECD guideline 416, groups of 30 male and 30 female Crl:CD®(SD)IGS BR rats were given diets containing chlorantraniliprole (purity, 96.45%) at a concentration of 0, 200, 1000, 4000, or 20 000 ppm, equal to 0, 12, 60, 238 and 1199 mg/kg bw per day in males and 0, 16, 78, 318, 1594 mg/kg bw per day in females of the P generation and equal to 0, 18, 89, 370, 1926 mg/kg bw per day in males and 0, 20, 104, 406 and 2178 mg/kg bw per day in females of the F_1 generation (corrected for purity). The rats were observed daily for clinical signs and detailed clinical observations were performed at least once per week. Body weights and food consumption were recorded weekly. Body weights and food consumption were also recorded on days 0, 7, 14 and 21 of gestation, and days 0, 7, 14, and 21 of lactation for the P and F_1 females. In P and F_1 rats, estrus cycle parameters (percentage of days in diestrus, proestrus, and estrus) and estrus cycle length were evaluated for 3 weeks before cohabitation. The age at either vaginal opening or preputial separation was recorded for the F_1 generation. Sperm motility, morphology and concentration in the cauda epididymis, and spermatid concentration in the testis were determined for P and F_1 rats. On postnatal day 4, litters were culled to eight pups. Until weaning at postnatal day 21, litters were examined for number of live and dead pups, pup weight and sex, clinical signs and external alterations on postnatal days 0, 4, 7, 14 and 21. After litter production, all P and F_1 parents were subjected to gross pathology and reproductive organs and brain, liver, spleen, adrenals, pituitary and kidneys were weighed. The reproductive organs, adrenal glands and gross lesions of all P and F_1 parents and F_2 weanlings and gross lesions of all weanlings were histologically examined. As adrenals were identified as potential target organs, adrenal glands from two males in the control group and in the group at the highest dose were examined by electron microscopy. Statements of adherence to QA and GLP were included.

There were no adverse, test substance-related effects on body weight, body-weight gain, food consumption, or food efficiency, clinical signs of toxicity, or mortality in P and F_1 males during pre-mating or in P and F_1 females during pre-mating, gestation, or lactation.

There were no test substance-related effects on sperm motility, morphology, epididymal sperm or testicular spermatid numbers in the P and F_1 males, nor on the mean percentage days in estrus, diestrus or proestrus, mean cycle length, or mean precoital interval in the P or F_1 females. Mating, fertility, duration of gestation, number of implantation sites, and implantation efficiency in the P and F_1 generations were not affected by chlorantraniliprole at any dietary concentration.

An increase in absolute and relative liver weights (up to 19%) was observed in P and F_1 females at 4000 ppm and above and was attributed to a pharmacological increase in metabolism. Livers were not examined microscopically in this study. In addition, an increase in mean absolute and relative adrenal weight (4–22%) was observed at 4000 and 20 000 ppm P and F_1 adults. A test substance-related increase in the number of rats displaying a minimal to mild increase in the degree of adrenal cortical microvesiculation was observed in P adult males at doses of 1000 ppm and above, in F_1 adult males at 200 ppm and higher, and in F_1 females at 20 000 ppm. Electron microscopy of the adrenal gland of two P males in the group at 20 000 ppm did not reveal any adverse, test substance-related effect.

Since there was no evidence of toxicologically adverse histological changes, no impact on adrenal function (assessed in a separate study), and adrenal weights were unaffected in other dietary studies in which rats were exposed for between 90 days and up to 2 years at similarly high concentrations, the effects on the adrenals were considered to be not adverse.

A transient small reduction in body weight (up to 9%) of the F_1 pups at 20 000 ppm on days 7, 14, and 21 of lactation had recovered by day 35 after weaning and was considered to be not adverse.

The NOAEL for parental toxicity, offspring toxicity and reproductive toxicity was 20 000 ppm, equal to 1199 mg/kg bw per day, the highest dose tested (Malley, 2006a).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, performed in accordance with OECD guideline 414, groups of 22 time-mated female Crl:CD®(SD)IGS BR rats were given chlorantraniliprole technical (purity, 96.45%) at a dose of 0, 20, 100, 300, or 1000 mg/kg bw per day by oral gavage in 0.5% aqueous methylcellulose on days 6–20 of gestation.

The rats were examined twice per day for clinical signs of toxicity. Body weight was recorded daily. Food consumption was recorded every other day. At termination on day 21 of gestation, the number of live and dead fetuses and fetal resorptions were recorded, live fetuses were weighed, sexed, and external alterations, intrauterine location and identification number were recorded. Approximately one half of the fetuses from each litter were examined for visceral abnormalities. During the external examination, all live fetuses with malformations were also examined for soft tissue alterations. All remaining live fetuses were examined for skeletal alterations. Dams were necropsied. Statements of adherence to QA and GLP were included.

No test substance-related effects on maternal clinical observations, body weight, body-weight gain, food consumption, or gross post-mortem observations were detected at any dose.

The mean number of corpora lutea, implantation sites, resorptions, live fetuses, fetal weight, and sex ratio were comparable in all groups. There were no abortions, premature deliveries, or complete litter resorptions and no effects of treatment on the numbers of litters, postimplantation loss, or on gravid uterine weights.

There were no test substance-related fetal external, visceral, or skeletal malformations or variations or adverse effects on fetal skeletal ossification observed at any dose.

The NOAEL for maternal and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Malley, 2004a).

Rabbits

In a study of developmental toxicity, performed in accordance with OECD guideline 414, groups of 22 time-mated Hra:(NZW)SPF female rabbits were given chlorantraniliprole (purity, 96.45%) at a dose of 0, 20, 100, 300, or 1000 mg/kg bw per day (corrected for purity) by gavage in 0.5% aqueous methylcellulose on days 7–28 of gestation. The rats were examined twice per day for clinical signs of toxicity. Body weight was recorded daily. Food consumption was recorded at 2–3 day intervals. At termination on day 29 of gestation, the number of live and dead fetuses and fetal resorptions were recorded, live fetuses were weighed, sexed, and external/visceral alterations and intrauterine location were recorded. All live fetuses were subsequently examined for skeletal alterations. Dams were necropsied. Statements of adherence to QA and GLP were included.

No test substance-related effects on maternal clinical observations, body weight, body-weight gain, food consumption, or gross post-mortem observations were detected in the does at any dose. The mean number of corpora lutea, implantation sites, resorptions, live fetuses, fetal weight, and sex

ratio were comparable in all groups. There were no abortions, premature deliveries, or complete litter resorptions and no effects of treatment on the numbers of litters, postimplantation loss, or on gravid uterine weights.

There were no test substance-related fetal external, visceral, or skeletal malformations or variations or adverse effects on fetal skeletal ossification observed at any dose.

The NOAEL for maternal and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Mylchreest, 2005).

2.5 Genotoxicity

Chlorantraniliprole was tested for genotoxicity in a range of guideline-compliant assays, both in vitro and in vivo. No evidence for genotoxicity was observed in any test. In addition to this core battery of studies of genetic toxicology, there was also a 2-week study in male and female rats dosed orally with chlorantraniliprole at 0, 25, 100, or 1000 mg/kg bw per day (see above; Munley, 2006a). Bone-marrow smears were prepared from rats in the main study and examined for the presence of micronuclei to assess potential genetic toxicity, and an additional group of rats was given cyclophosphamide as a positive control for genetic toxicity. Reportedly, no increases in the micronucleated PCEs in the ratio of PCEs/NCEs were observed in any evaluated test substance-treated group of male or female rats (data not presented).

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

2.6 Special studies

(a) Neurotoxicity

In a study of acute neurotoxicity, performed in accordance with OPPTS guideline 870.6200, groups of 12 male and 12 female Crl:CD®(SD)IGS BR rats were given chlorantraniliprole (purity, 95.9%) as a single dose at 0, 200, 700, or 2000 mg/kg bw (corrected for purity) by gavage in 0.5%

Table 3. Results of studies of genotoxicity with chlorantraniliprole

End-point	Test object	Concentration/dose	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537, *E. coli* WP2 *uvr*A	2.5–5000 µg/plate ± S9[a]	96.45	Negative	Wagner & Atta-Safoh (2004)
Gene mutation	CHO-K_1 cells, HGPRT test	15.6–250 µg/ml[b] ± S9[a]	96.45	Negative	San & Clarke (2004)
Chromosomal aberration	Human lymphocytes	125–500 µg/ml[c] ± S9	96.45	Negative	Gudi & Rao (2004)
In vivo					
Micronucleus formation	Mouse bone marrow	500–2000 mg/kg bw (gavage)	96.45	Negative	Donner (2006b)

Positive and negative (solvent) controls were included in all studies. In all studies, statements of adherence to GLP and QA were included.

[a] Precipitation at ≥ 1800 µg/plate

[b] Precipitation at 250 µg/ml

[c] Precipitation at 500 µg/ml

methylcellulose. The rats were checked daily for clinical signs of toxicity. Body weight and food consumption were recorded on days 1 (before treatment), 2, 8 and 15. A neurobehavioral test battery, consisting of motor activity and FOB assessments, was conducted on all rats before dosing, approximately 2 h after dosing on day 1, and on days 8 and 15. At termination on days 16 and 17, six male and six female rats were examined grossly. The central and peripheral nervous system and selected muscle tissues of the six males and six females from all groups were collected, and microscopic neuropathological evaluations were conducted on rats in the control group and at the highest dose. Statements of adherence to QA and GLP were included.

No adverse compound-related effects on mortality, clinical signs of toxicity, body weight, body-weight gain, food consumption, food efficiency, FOB parameters, motor activity, gross pathology, or neuropathology were observed at any dose in males or females.

The NOAEL was 2000 mg/kg bw, the highest dose tested (Malley, 2004b).

In a 90-day study of neurotoxicity, performed in accordance with OPPTS guideline 870.6200 (resembles OECD guideline 424), groups of 12 male and 12 female Crl:CD®(SD)IGS BR rats were given diets containing chlorantraniliprole (purity, 96.45%) at a concentration of 0, 200, 1000, 4000, or 20 000 ppm, equal to 0, 13, 64 and 255 mg/kg bw per day in males and 0, 15, 77 and 255 mg/kg bw per day in females (corrected for purity). The rats were checked twice per day for clinical signs of toxicity. A detailed physical examination was performed weekly. Body weight and food consumption were recorded weekly and on the days of FOB and motor activity testing. Assessments of FOB and motor activity were conducted on all rats before exposure (baseline) and during weeks 4, 8, and 13. At termination, six males and six females per group were examined grossly. The central and peripheral nervous system and selected muscle tissues of the six males and six females rats from all groups were collected, and microscopic neuropathological evaluations were conducted on rats in the control group and in the group at the highest dose. Statements of adherence to QA and GLP were included.

There were no test substance-related effects on mortality, clinical observations, body weight, body-weight gain, food consumption, food efficiency, FOB parameters, motor activity, or on gross or microscopic pathology in males or females.

The NOAEL was 20 000 ppm, equal to 1313 mg/kg bw per day, the highest dose tested (Malley, 2006b).

(b) Immunotoxicity

In a 28-day study of immunotoxicity, performed in accordance with OPPTS guideline 870.7800, groups of 10 male and 10 female Crl:CD-1®(ICR)BR mice were given diets containing chlorantraniliprole technical (purity, 96.45%) at a concentration of 0, 300, 1700, or 7000 ppm, equal to 0, 48, 264, or 1144 mg/kg bw per day for males and 0, 64, 362, or 1566 mg/kg bw per day for females (corrected for purity). The mice were checked daily for mortality and morbidity and weekly for clinical signs. Body weight and food consumption were recorded weekly. On day 23, the mice were injected intravenously with 0.2 ml of 1×10^9 sheep erythrocytes/ml. On day 28, sheep erythrocyte-specific IgM concentrations in blood were measured. Each mouse was examined grossly and the thymus, spleen, and brain were weighed. Statements of adherence to QA and GLP were included.

No treatment-related effects on body weight, food consumption, gross pathology, organ weight, or sheep erythrocyte-specific antibody (IgM) response were observed.

The NOAEL was 7000 ppm, equal to 1144 mg/kg bw per day, the highest dose tested (Munley, 2007).

In a 28-day study of immunotoxicity, performed in accordance with OPPTS guideline 870.7800, groups of 10 male and 10 female Crl:CD®(SD)IGS BR rats were fed diets containing chlorantra-

niliprole technical (purity, 96.45%) at a concentration of 0, 1000, 5000, or 20 000 ppm, equal to 0, 74, 363, or 1494 mg/kg bw per day for males and 0, 82, 397, or 1601 mg/kg bw per day for females (corrected for purity). The rats were checked daily for mortality and morbidity and weekly for clinical signs of toxicity. Body weight and food consumption were recorded weekly. On day 22, the rats were injected intravenously with 0.5 ml of 4×10^8 sheep erythrocytes/ml. On day 28, sheep erythrocyte-specific IgM concentrations in blood were measured. Each rat was examined grossly and the thymus, spleen, and brain were weighed. Statements of adherence to QA and GLP were included.

No treatment-related effects on body weight, food consumption, gross pathology, organ weight or sheep erythrocyte-specific antibody (IgM) response were observed.

The NOAEL was 20 000 ppm, equal to 1494 mg/kg bw per day, the highest dose tested (Munley, 2006b).

(c) Studies with metabolites

The rat metabolite IN-EQW78 is also a significant metabolite in soil, water, and sediment. The substances IN-ECD73 and IN-F6L99 are metabolites that are only observed at low concentrations in soil and as degradates in studies of high-temperature food processing.

(i) Acute toxicity

The results of studies of acute toxicity with metabolites of chlorantraniliprole are summarized in Table 4. No substance-related clinical signs of toxicity were observed in the studies of acute toxicity.

Table 4. Acute toxicity with metabolites of chlorantraniliprole

Species	Strain	Sex	Route	Metabolite	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	Crl:CD-1®(ICR) BR	Female	Oral	IN-ECD73[a]	99.8	> 2000	Finlay (2006e)[b,c]
Rat	Crl:CD®(SD)IGS BR	Female	Oral	IN-EQW78[d]	99.8	> 2000	Finlay (2006f)[b,c]
Mouse	Crl:CD-1®(ICR) BR	Female	Oral	IN-F6L99[e]	99.8	> 2000	Finlay (2006g)[b,c]

[a] IN-ECD73: 2,6-Dichloro-4-methyl-11*H*-pyrido[2,1-b]quinazolin-11-one.
[b] Performed according to the up-and-down procedure, OECD guideline 425.
[c] Statements of adherence to good laboratory practice and quality assurance were included.
[d] IN-EQW78: 2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-3,8-dimethyl-4(3*H*)-quinazolinone.
[e] IN-F6L99: 3-Bromo-*N*-methyl-1*H*-pyrazole-5-carboxamide

Table 5. Results of studies of genotoxicity with metabolites of chlorantraniliprole

Metabolite	End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro						
IN-EQW78[a]	Reverse mutation	*S. typhimurium.* strains TA98, TA100, TA1535 and TA1537, and *E. coli* WP2 *uvr*A	0–3333 µg/ plate ± S9	99.8	Negative	Ford (2006)
IN-ECD73[b]	Reverse mutation	*S. typhimurium.* strains TA98, TA100, TA1535 and TA1537, and *E. coli* WP2 *uvr*A	0–5000 µg/ plate ± S9	99.8	Negative	Myhre (2006a)
IN-F6L99[c]	Reverse mutation	*S. typhimurium.* strains TA98, TA100, TA1535 and TA1537, and *E. coli* WP2 *uvr*A	0–5000 µg/ plate ± S9	98.6	Negative	Myhre (2006b)

Positive and negative (solvent) controls were included in all studies. In all studies, statements of adherence to GLP and QA were included.
[a] IN-EQW78: 2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-3,8-dimethyl-4(3*H*)-quinazolinone.
[b] IN-ECD73: 2,6-Dichloro-4-methyl-11*H*-pyrido[2,1-b]quinazolin-11-one.
[c] IN-F6L99: 3-Bromo-*N*-methyl-1*H*-pyrazole-5-carboxamide

(ii) Genotoxicity

The results of studies of genotoxicity with metabolites of chlorantraniliprole are summarized in Table 5. The Meeting concluded that these metabolites of chlorantraniliprole are unlikely to be genotoxic.

3. Observations in humans

Chlorantraniliprole has at present only been produced on a pilot scale. In the limited number of workers involved with the synthesis of this compound to date, no illnesses have been attributed to exposure associated with the handling, testing, or manufacturing of chlorantraniliprole.

Comments

Biochemical aspects

After oral administration, the extent of absorption of chlorantraniliprole is dependent on the dose administered. At a single dose of 10 mg/kg bw, absorption was about 73–85%, with 18–30% being excreted in the urine and 49–53% being excreted in the bile within 48 h. At a single dose of 200 mg/kg bw, absorption was about 14%, with 4% and 5–7% of the dose excreted in the urine and bile, respectively, within 48 h. Excretion in expired air was insignificant. Plasma half-lives were 38–43 h in males and 78–82 h in females. After multiple doses (10 mg/kg bw per day for 14 days) with chlorantraniliprole, peak plasma concentrations in males and females were about two and seven times higher than after a single dose at 10 mg/kg bw, respectively. Distribution in tissues was extensive, with 0.8% and 3% remaining in the tissues of males and females, respectively, 168 h after a single dose at 10 mg/kg bw.

Chlorantraniliprole is extensively metabolized through tolyl methyl and *N*-methyl carbon hydroxylation, followed by *N*-demethylation, nitrogen-to-carbon cyclization with loss of a water molecule resulting in the formation of the pyrimidone ring, oxidation of alcohols to carboxylic acids, amide-bridge cleavage, amine hydrolysis, and *O*-glucuronidation. The potential for hydroxylation of the tolyl methyl and *N*-methyl carbon groups was greater in males than in females. After a single dose at 200 mg/kg bw, excretion of the parent compound in the urine and faeces (78.9–85.5%) was 12 to16-fold that at 10 mg/kg bw (4.9–7.3%). The profile of metabolites after a single dose at 200 mg/kg bw or after repeated doses at 10 mg/kg bw per day was similar to the profile after a single dose at 10 mg/kg bw.

Toxicological data

The acute toxicity of chlorantraniliprole is low (oral and dermal LD_{50}, > 5000 mg/kg bw; inhalation LC_{50}, > 5.1 mg/l). Apart from ocular and nasal discharge observed in a study in which chlorantraniliprole was administered by inhalation, no clinical signs of toxicity were observed in studies of acute toxicity. Chlorantraniliprole is not irritating to the skin and eyes, and is not a skin sensitizer (Magnussen & Kligman test in guinea-pigs; local lymph node assay in mice).

Chlorantraniliprole shows low toxicity after repeated doses. Occasionally, reductions in body-weight gain were observed in studies with repeated doses. However, these reductions often did not occur on consecutive weeks but were seen sporadically, were not dose-related and were not consistently found in different studies at similar or higher doses. Therefore, the incidental changes in body-weight gain were not considered to be a compound-related effect.

In short-term studies with chlorantraniliprole administered orally (gavage or diet), no adverse effects were observed at any dose tested, i.e., up to 7000 ppm, equal to 1443 mg/kg bw per day, in feeding studies in mice, up to 20 000 ppm, equal to 1188 mg/kg bw per day, in a feeding study in rats, and up to 40 000 ppm, equal to 1164 mg/kg bw per day, in a 1-year feeding study in dogs.

In an 18-month feeding study in mice, the NOAEL was 1200 ppm, equal to 158 mg/kg bw per day, on the basis of presence of eosinophilic foci in the liver, accompanied by hepatocellular hypertrophy and increased liver weight at 7000 ppm, equal to 935 mg/kg bw per day, in males only. No information on the chemical-specific mechanism of action was available to evaluate the relevance of liver foci to exposure of humans. However, the Meeting noted that this is a possible species- and sex-specific response that is of questionable toxicological significance and relevance, and thus the NOAEL of 158 mg/kg bw per day on the basis of these end-points is likely to be conservative.

In a 2-year feeding study in rats, the NOAEL was 20 000 ppm, equal to 805 mg/kg bw per day, the highest dose tested.

No treatment-related changes in the incidence of tumours were observed.

The Meeting concluded that chlorantraniliprole is not carcinogenic in rodents.

Chlorantraniliprole was tested for genotoxicity in adequate range of studies of genotoxicity in vitro and in vivo. No evidence for genotoxicity was observed in any test. The Meeting concluded that chlorantraniliprole is unlikely to be genotoxic.

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

In a two-generation study of reproductive toxicity with chlorantraniliprole in rats, the NOAEL for parental, offspring and reproductive toxicity was 20 000 ppm, equal to 1199 mg/kg bw per day, the highest dose tested.

In a study of developmental toxicity in rats, the NOAEL for maternal and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested. In a study of developmental toxicity in rabbits, the NOAEL for maternal and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

In a study of acute neurotoxicity in rats given chlorantraniliprole orally by gavage, the NOAEL was 2000 mg/kg bw per day, the highest dose tested. In a 90-day dietary study of neurotoxicity in rats, the NOAEL was 20 000 ppm, equal to 1313 mg/kg bw per day, the highest dose tested.

In a dietary study of immunotoxicity in mice, the NOAEL was 7000 ppm, equal to 1144 mg/kg bw per day, the highest dose tested. In a dietary study of immunotoxicity in rats, the NOAEL was 20 000 ppm, equal to 1494 mg/kg bw per day, the highest dose tested.

To date, chlorantraniliprole has only been produced on a pilot scale. In the limited number of workers involved with the synthesis of this compound to date, no illnesses have been attributed to exposure associated with the handling, testing, or manufacturing of chlorantraniliprole.

The rat metabolite 2-[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-3,8-dimethyl-4(3*H*)-quinazolinone (IN-EQW78) was also a significant metabolite in soil, water, and sediment. The substances 2,6-dichloro-4-methyl-11*H*-pyrido[2,1-*b*]quinazolin-11-one (IN-ECD73) and 3-bromo-*N*-methyl-1*H*-pyrazole-5-carboxamide (IN-F6L99) were metabolites only observed at low concentrations in soil and as degradates in studies of high-temperature food processing. In studies of acute toxicity, these three chlorantraniliprole metabolites had LD_{50}s of > 2000 mg/kg bw. These metabolites gave negative results in a test for reverse mutation.

The Meeting concluded that the existing database on chlorantraniliprole is sufficient to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) for chlorantraniliprole of 0–2 mg/kg bw on the basis of eosinophilic foci accompanied by hepatocellular hypertrophy and increased liver weight in mice in an 18-month feeding study for which the NOAEL was 158 mg/kg bw per day, and using a safety factor of 100. There was no available information on the chemical-specific mechanism

of action with which to evaluate the relevance of the liver foci to exposure of humans. The Meeting noted, however, that this is a possible species- and sex-specific response that is of questionable toxicological significance and relevance, and thus the NOAEL of 158 mg/kg bw per day (and consequently the ADI) identified on the basis of these end-points is likely to be conservative.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for chlorantraniliprole in view of its low acute toxicity, the absence of developmental toxicity, and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

Levels relevant for risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month study of toxicity and carcinogenicity[a]	Toxicity	1200 ppm, equal to 158 mg/kg bw per day	7000 ppm, equal to 935 mg/kg bw per day
		Carcinogenicity	7000 ppm, equal to 935 mg/kg bw per day[c]	—[c]
Rat	Two-year study of toxicity and carcinogenicity [a]	Toxicity	20 000 ppm, equal to 805 mg/kg bw per day	—[c]
		Carcinogenicity	20 000 ppm, equal to 805 mg/kg bw per day[c]	—[c]
	Two-generation study of reproductive toxicity[a]	Parental	20 000 ppm, equal to 1199 mg/kg bw per day	—[c]
		Offspring toxicity	20 000 ppm, equal to 1199 mg/kg bw per day	—[c]
		Reproductive toxicity	20 000 ppm, equal to 1199 mg/kg bw per day	—[c]
	Developmental toxicity[b]	Maternal toxicity	1000 mg/kg bw per day	—[c]
		Foetotoxicity	1000 mg/kg bw per day	—[c]
	Acute neurotoxicity[b]	Neurotoxicity	2000 mg/kg bw per day	—[c]
	90-day neurotoxicity[a]	Neurotoxicity	20 000 ppm, equal to 1313 mg/kg bw per day	—[c]
Rabbit	Developmental toxicity[b]	Maternal toxicity	1000 mg/kg bw per day	—[c]
		Foetotoxicity	1000 mg/kg bw per day	—[c]
Dog	One-year study[a]	Toxicity	40 000 ppm, equal to 1164 mg/kg bw per day	—[c]

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

Estimate of acceptable daily intake for humans

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 chlorantraniliprole

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of absorption	Rapid, incomplete and dose-dependent oral absorption (73–85% at 10 mg/kg bw; 14% at 200 mg/kg bw).
Distribution	Extensive (rats)
Potential for accumulation	Low in males, moderate in females (rats)
Rate and extent of excretion	Plasma half-lives: males, 38–43 h; females, 78–82 h
	At 10 mg/kg bw: 18–30% in urine, 49–53% in bile, within 48 h.
	At 200 mg/kg bw: 4% in the urine, 5–7% in bile, within 48 h.
Metabolism in animals	Extensive, through tolyl methyl and *N*-methyl carbon hydroxylation, followed by *N*-demethylation, nitrogen-to-carbon cyclization, formation of a pyrimidone ring, oxidation of alcohols to carboxylic acids, amide-bridge cleavage, amine hydrolysis, and *O*-glucuronidation.
Toxicologically significant compounds (animals, plants and environment)	Chlorantraniliprole
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.1 mg/l
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Dermal sensitization	Not sensitizing (Magnussen & Kligman test in guinea-pigs; local lymph node assay in mice)
Short-term studies of toxicity	
Target/critical effect	None
Lowest relevant oral NOAEL	1443 mg/kg bw per day (mice), 1188 mg/kg bw per day (rats), 1164 mg/kg bw per day (dogs); highest doses tested
Lowest relevant dermal NOAEL	1000 mg/kg bw per day, the highest dose tested (rat)
Lowest relevant inhalatory NOAEC	No data available
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver: eosinophilic foci, hepatocellular hypertrophy, increased liver weight (mice)
Lowest relevant NOAEL	1200 ppm, equal to 158 mg/kg bw per day (mice)
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	20 000 ppm, equal to 1199 mg/kg bw per day, the highest dose tested (rats)
Developmental target	No developmental effects (rats, rabbits)
Lowest relevant developmental NOAEL	1000 mg/kg bw per day, the highest dose tested (rats, rabbits)
Neurotoxicity/delayed neurotoxicity	
Neurotoxicity	No neurotoxic effects
Lowest relevant oral NOAEL	2000 mg/kg bw, the highest dose tested (acute toxicity in rats treated by gavage)
	1313 mg/kg bw per day, the highest dose tested (90-day dietary study in rats)

Other toxicological studies	
Immunotoxicity	Not immunotoxic
Lowest relevant oral NOAEL	7000 ppm, equal to 1144 mg/kg bw per day, the highest dose tested (28-day study in mice)
	20 000 ppm, equal to 1494 mg/kg bw per day, the highest dose tested (28-day study in rats)
Medical data	
	No adverse effects observed in workers involved with the synthesis of this compound

Summary

	Value	*Study*	*Safety factor*
ADI	0–2 mg/kg bw	Mouse, 18-month study	100
ARfD	Unnecessary	—	—

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HEXYTHIAZOX

First draft prepared by
I. Dewhurst,[1] & A. Boobis[2]

[1] Pesticides Safety Directorate, York, England; and
[2] Imperial College London, London, England

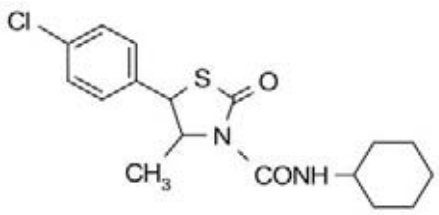
Explanation

Hexythiazox is the International Organization for Standardization (ISO) approved name for (*trans*-5-(4-chlorophenyl)-*N*-cyclohexyl-4-methyl-2-oxo-3-thiazolidine-carboxamide (CAS No. 78587-05-0). Hexythiazox is an acaricide that acts against egg, larval and nymph stages. The precise mechanism of acaricidal action is unknown.

Hexythiazox was evaluated previously by the JMPR in 1991 when an acceptable daily intake (ADI) of 0–0.03 mg/kg bw was established based on a no-observed-adverse-effect level (NOAEL) of 3.2 mg/kg bw per day identified in a 2-year study in rats and with a safety factor of 100. Hexythiazox was reviewed by the present Meeting as part of the Codex Committee on Pesticide Residues (CCPR) periodic review programme. Two additional studies of genotoxicity and some revised study reports were available to the present Meeting.

Most of the pivotal studies met the basic requirements of the relevant Organization for Economic Co-operation and Development (OECD) or national test guidelines. Only a small number of study reports contained certificates of compliance with good laboratory practice (GLP).

Figure 1. Chemical structure of hexythiazox



Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

Rats

Three groups of five male and five female Fischer F344 rats (age 10 weeks) rats were given a single oral dose of [thiazolidine-5-^{14}C]hexythiazox (specific activity, 6.6 mCi/mmol; purity, > 99%). The three different treatment protocols included a single oral dose at a lower dose (10 mg/kg bw), a single oral dose at the lower dose after fourteen oral doses of unlabeled hexythiazox and a single oral dose at a higher dose (880 mg/kg bw). The lower dose equated to approximately 50 µCi/kg bw and the higher dose, approximately 90 µCi/kg bw. The vehicle chosen for administration of the lower dosedimethyl sulfoxide (DMSO), while the vehicle chosen for the higher dose was olive oil, because of the solubility limitations of hexythiazox in DMSO. Blood and excreta were collected regularly over the 3 or 4 days after dosing. Concentrations of radioactivity were determined in fifteen tissues, in addition to plasma, and digestive organs and their contents. Expired gases were not collected because no volatile radioactivity (less than 0.01%) was recovered in a preliminary study. Analyses of the metabolites in urine and faeces were carried out on days 0–1 and 1–2 only, because subsequent samples contained only low levels of radioactivity. The metabolites of hexythiazox in excreta were determined by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). In a preliminary experiment, faecal metabolite identification was performed using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). The levels of bound ^{14}C-residues in tissues were evaluated by polar solvent extraction. The study protocols are outlined in Table 1.

The maximum concentrations of radioactivity in plasma were observed about 3–4 h after administration in the groups at 10 mg/kg bw groups (groups B & C) and 12 h after dosing at at 880 mg/kg bw (group D). At those times the mean plasma concentrations of radioactivity in groups B and C were 1.8–2.2 ppm for males and 2.3–2.6 ppm for females, and in group D, 37 ppm for males and 27 ppm for females; indicating saturation of absorption at the higher dose. At 72 or 96 h after administration approximately 0.1 ppm of the radioactivity remained in the plasma in groups B and C; the concentrations of radioactivity in plasma in group D had decreased to approximately 2 ppm. The plasma absorption and elimination followed first-order kinetics, with rate constants of 0.53 h^{-1} and approximately 0.075 h^{-1} respectively, at 10 mg/kg bw in males. The elimination rate constant

Table 1. Design of a study of the absorption, distribution, metabolism and excretion of radiolabelled hexythiazox in rats treated orally

Parameter	Preliminary group	Group B	Group C	Group D
Nominal dose (mg/kg bw)	10	10	10 (after 14 days of pre-treatment with non-labelled hexythiazoz at 10 mg/kg bw per day)	880
No. of rats (male/female)	3/0	5/5	5/5	5/5
Duration (h)	48	72	96	96
Samples	Exhaled air, urine, faeces	Urine, faeces, blood, tissues	Urine, faeces, blood, tissues	Urine, faeces, blood, tissues
Methods of analysis	Total ^{14}C	Total ^{14}C TLC, HPLC, NMR, MS	Total ^{14}C TLC, HPLC	Total ^{14}C- TLC, HPLC

From Soeda (1983); Soeda (1985a, 1985b, 1985c, 1985d)

HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; MS, mass spectrometry; TLC, thin-layer chromatography;

corresponded to a half-life of approximately 9 h. In females, the half-life was slightly longer at 11.4 h and the half-life was also prolonged in males and females at the higher dose, at 17.3 and 21.7 h, respectively.

In groups B and C approximately 30% of the administered radioactivity was excreted in the urine and approximately 60–70% of the administered radioactivity was recovered in the faeces. In group D, 9.5% of the radioactivity was found in the urine and 89.1% in the faeces. Of the administered dose, 1.1–10.1% was associated with tissues at 96 h. The highest concentrations of radioactivity were found in fat, adrenal, liver, ovary and digestive organs and their contents. The highest concentrations of radioactivity in tissues were observed in fat 96 h after dosing, reaching approximately 2.3, 1.2 and 76 ppm in males and 5.4, 3.3 and 129 ppm in females, in groups B, C and D, respectively. Approximately 36–71% of the radioactivity in the liver and less than 2% of the radioactivity in fat remained as bound ^{14}C after extraction. Residue concentrations in fat were generally twice as high in females as in males. Concentrations of radioactivity in fat at the end of the studies were low, but more than 20fold those in plasma, indicating some potential for bioaccumulation. There were no remarkable differences in patterns of absorption and excretory patterns between males and females or after repeated doses (Soeda, 1983; Soeda, 1985a, 1985b, 1985c, 1985d).

The dermal penetration of hexythiazox has been investigated in rats in vivo. Groups of eight male Crl:CD(SD)BR rats were exposed to ^{14}C-hexythiazox in aqueous suspensions of a wettable powder formulation at three different dilutions. The application volume was 200 µl/25 cm^2. Four rats per group were fitted with jugular-vein cannulae and were exposed for 10 h, during which time samples of blood and excreta were collected and analysed by liquid scintillation counting (LSC). The remaining four rats per group were exposed for 1 h and samples of excreta were collected. At termination (at 10 h or 1 h in cannulated and non-cannulated rats, respectively) the concentration of radioactivity in a range of samples was determined by LSC. After an initial rapid absorption phase between 0 h and 0.5 h, absorption was linear, showing no diminution at the end of the 10 h exposure period. Only a small proportion of the applied radioactivity was absorbed (< 2%) in cannulated

rats. The shortcomings of this study, in terms of exposure time for the non-cannulated rats, no measurements taken more than 10 h after the start of exposure and no information on the residue at the application site, do not permit a definitive determination of the dermal penetration of hexythiazox to be made from this study (Grube, 1986).

1.2 Biotransformation

Rats

Samples from the studies of Soeda (1983) and (1985a, 1985b, 1985c, 1985d) (see above) were extracted and analysed for metabolites. In the groups at the lower dose, the primary identified compound was hexythiazox, present in the urine at approximately 2% of the administered radioactivity and in faeces at approximately 40% of the administered radioactivity. Unidentified compounds extracting into methanol represented approximately 80% of the radioactivity in urine and 30% of the faecal radioactivity. The primary, identified metabolic reactions were hydroxylation of the cyclohexane ring and cleavage of the amide-cyclohexane bond. The major identified radiolabelled metabolite in excreta was PT-1-8 (*cis*), which comprised 8–12% of the administered radioactivity in excreta in the groups at the lower dose. The remaining identified metabolites were present at low concentrations (each < 2% of the administered radioactivity): PT-1-2, PT-1-3, PT-1-4, PT-1-8 (*trans*), PT-1-9, PT-1-10 and PC-1-1 (see Figure 1 for metabolite names and structures). The major identified radiolabelled component in fat was the parent compound; the predominant metabolite in liver and kidney was PT-1-4. With such a high proportion of unidentified material, the metabolism of hexythiazox in rats cannot be considered to have been fully characterized (Gomyo, 1991).

The proposed metabolic pathway of hexythiazoz is given in Figure 1.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Hexythiazox was of low acute toxicity when administered orally, dermally or by inhalation. No deaths were seen in any of the submitted studies (Table 2).

Table 2. Acute toxicity of hexythiazox

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Purity (%)	Reference
Rat	SLC:SD	Males & females	Oral	> 5000 (water + Tween 80)	—	98.3	Saika et al. (1983a)
Mouse	SLC:ICR	Males & females	Oral	> 5000 (water + Tween 80)	—	98.3	Saika et al. (1983b)
Dog	Beagle	Males & females	Oral	> 5000 (water + Tween 80)	—	98.3	Saika et al. (1984)
Rat	SLC:SD	Males & females	Dermal	> 5000 (water + Tween 80)	—	98.3	Saika et al. (1983c)
Rat	SLC:SD	Males & females	Inhalation (4 h, whole body)	—	> 2.0 [a] (dust aerosol; MMAD, 4.2 µm)	92.3	Saika et al. (1983d)

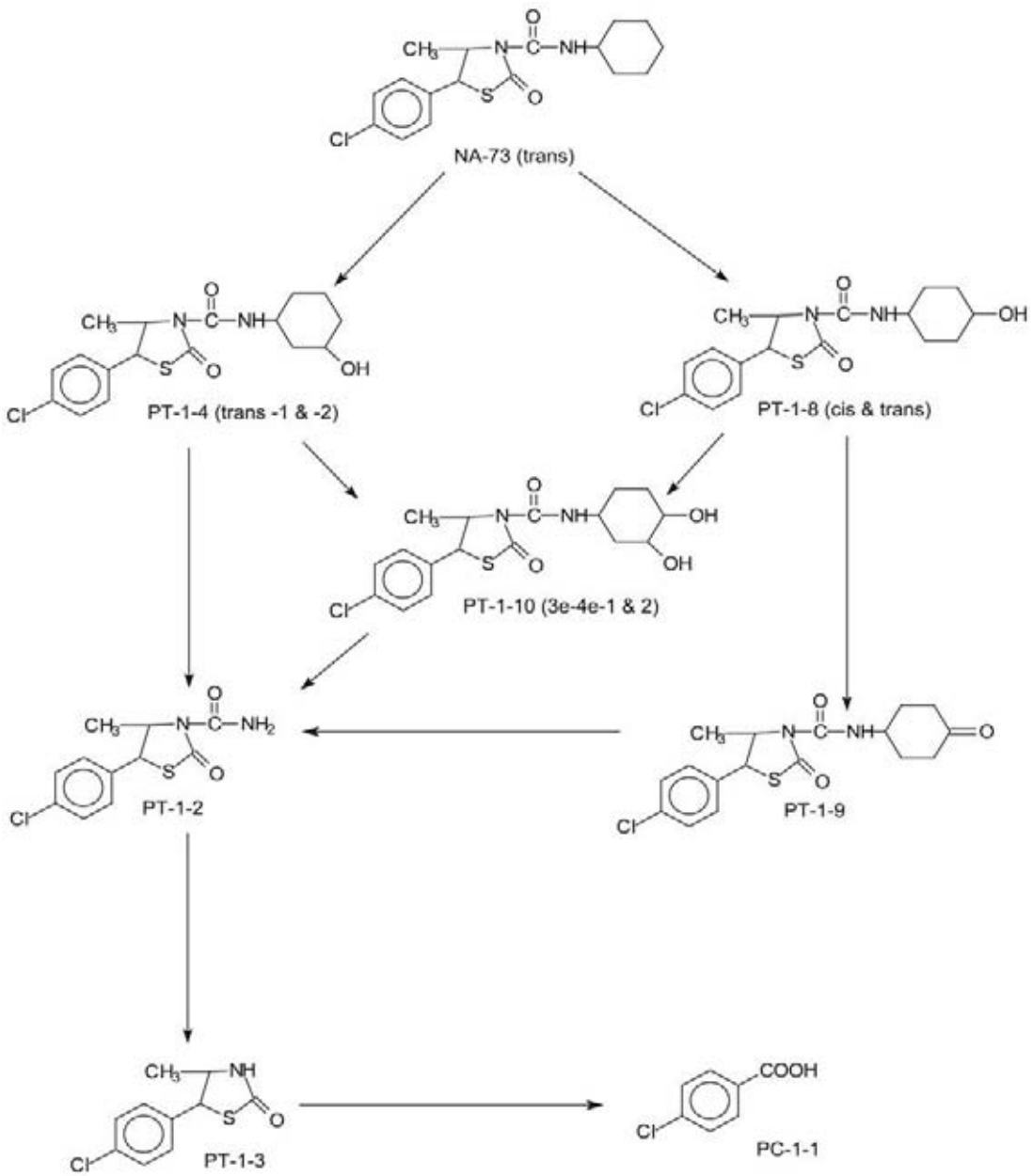
MMAD, mass median aerodynamic diameter.

[a] Maximal technically achievable concentration.

(b) Dermal and ocular irritation and dermal sensitization

Hexythiazox was not irritating to the skin of rabbits (Souma et al., 1983a); was a slight, transient eye irritant (Souma et al., 1983b) and produced no evidence of skin sensitizing potential in maximization tests (Souma et al., 1983c; Takaori, 2006).

Figure 2. Proposed metabolic pathway of hexythiazox in the rat



NA-73: hexythiazox
PC-1-1: 4-chlorobenzoic acid
PT-1-2: 5-(4-chlorophenyl)-4-methyl-2-oxo-3-thiazolidine-carboximide
PT-1-3: 5-(4-chlorophenyl)-4-methyl-2-oxo-3-thiazolidine
PT-1-4: 3-hydroxy hexythiazox
PT-1-8: 4-hydroxy hexythiazox
PT-1-9: 4-oxo hexythiazox
PT-1-10: 3,4-dihydroxy hexythiazox

2.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female B6C3F$_1$ mice (age 6 weeks) were given diets containing hexythiazox (purity, 98.3%) at a concentration of 0, 50, 300, 1800 or 10 800 ppm for 28 days. Diets were supplied biweekly and stored in a freezer (–20 °C) until used. Haematological tests and clinical chemistry analyses were carried out at termination of the study. Urine samples were collected during week 3. Gross post-mortem examinations were performed, organ weights were determined and histopathological examination of liver, kidneys, heart, lung and spleen was performed on all mice. Histopathological evaluation of a wide range of tissues was conducted for mice in the control group and mice at the highest dose.

The homogeneity and content of the diet were not confirmed analytically. Mean daily intake of hexythiazox was reported as 10/13; 55/63; 319/388 and 1908/2045 mg/kg bw per day for males and females in the four treatment groups, respectively.

A male mouse in the group at 50 ppm died from a bite wound at 22 days. Adverse clinical signs were not observed in any of the mice treated with hexythiazox. Body-weight gain was decreased significantly (approximately 15%) at 3 and/or 4 weeks in males at 50, 1800 and 10 800 ppm, but not at 300 ppm. There was no notable change in food consumption. Total cholesterol was decreased significantly in males and females at 10 800 ppm (30–40%) and in males at 1800 ppm (approximately 20%). Specific gravity of urine was increased in males at 10 800 ppm. Enzyme markers of liver toxicity were similar in rats receiving hexythiazoz and rats in the control groups. A dose-dependent increase in liver weight and liver/body weight ratio was observed at 1800 ppm (approximately 10%) and 10 800 ppm (approximately 30%), in males and females. Swollen centrilobular liver cells were observed in the majority of males and females at 10 800 and in males at 1800 ppm. These cells were characterized by slightly enlarged nuclei and eosinophilic cytoplasm, not stainable by periodic acid-Schiff (PAS) stain. Fat droplets, which were usually visible in the liver of rats in the control group, were not apparent in these swollen cells.

The NOAEL was 300 ppm, equal to 55 mg/kg bw per day, on the basis of decreased body-weight gain and decreased total cholesterol in males at 1800 ppm (Takaori et al., 1983a).

Rats

Groups of 20 male and 20 female Fischer F344 rats were given diets containing technical hexythiazox (purity, 98.3%) at a concentration 0, 10, 70, 500 or 3500 ppm for 90 days. Satellite groups of 20 males and 20 females were used for interim measurements. An additional group of 20 males and 20 females was used for determination of baseline values for haematology, blood chemistry and cholinesterase activity. Haematological tests were performed pre-test and at 1.5 and 3 months. Blood chemistry determinations were performed pre-test and at 2 and 3 months. Cholinesterase activities (brain, plasma and erythrocyte) were determined after overnight fasting at 0, 1, 2 and 3 months, in 10 males and 10 females. Urine analysis was performed after 1.5 and 3 months. Gross necropsy and histopathology were performed on a wide range of tissues from all rats.

The homogeneity and content of the diet were confirmed analytically. Mean daily intake of hexythiazox was 0.7/0.8, 4.9/5, 36/38 and 266/258 mg/kg bw per day for males and females, respectively.

All rats survived until study termination without any clinical signs of toxicity. Body weight and body-weight gain were reduced in males and females at 3500 ppm and females at 500 ppm. Body-weight gain was reduced in males and females at 3500 ppm for almost the entire study period, starting from around week 4, and slightly in females at 500 ppm from week 11 (Table 3). There was a slight reduction (< 10%) in food consumption in males and females at 3500 ppm; on occasional weeks, the

reduction was statistically significant compared with values for the control group, but there was no obvious trend with duration of treatment. Food efficiency in females at 3500 ppm and 500 ppm was decreased at 1 week of feeding.

A number of haematological changes were seen in males at 3500 ppm (Table 3). A reduction in leukocyte count and effects on urine analysis results at 1.5 months, but not at 3 months, were not considered to be adverse findings given the absence of consistency over time. Significant changes in clinical chemistry results were seen in males and females at 3500 ppm; minor changes observed in rats at 500 ppm were not considered to be adverse (Table 3). Plasma cholinesterase activity was statistically significantly decreased at 500 (12 and 13% at 2 and 3 months, respectively) and 3500 ppm (10%, 25% and 24% at 1, 2 and 3 months, respectively) in females, but erythrocyte and brain acetylcholinesterase activities were not inhibited.

Absolute liver (approximately 8% and approximately 40%, respectively) and relative liver/body weights (approximately 10% and approximately 47%, respectively) were increased in males and females at 3500 and 500 ppm (Table 4). At 3500 ppm, absolute spleen weight and spleen/body weight ratio were decreased in males and females, kidney/body weight, adrenal/body weight and gonad/body-weight ratios were increased in males and females, absolute adrenal weight was increased in males, absolute thymus weight was decreased in females and brain/body-weight ratio was increased in females (Table 4).

All males at 3500 ppm suffered from glomerulonephrosis and 60% of males (12 out of 20) in all other groups, including controls. Hepatocellular hypertrophy was observed in all males and females at 3500 ppm. Fatty degeneration of adrenal cortex (zona fasciculate) was found in all males and 65% (13 out of 20) of females at 3500 ppm and in all males and 20% (4 out of 20) of females at 500 ppm versus zero in the control group and the groups at the lower doses.

The NOAEL was 70 ppm, equal to 4.9 mg/kg bw per day, on the basis of reduced body-weight gain, and increased fatty degeneration of the adrenal cortex (Takaori et al., 1983b).

Dogs

Groups of two male and two female beagle dogs were given diets containing hexythiazox (purity, 97.7%) at a concentration of 125, 500, 2000 or 8000 ppm for 4 weeks. Dogs in the control group received basal diet. The weights of brain, liver, kidney, heart, spleen, testis, ovary, pituitary, thyroid/parathyroid and adrenal were recorded. However, only liver and adrenal samples were prepared and examined microscopically.

The homogeneity and content of the diet were not confirmed analytically. Mean daily intakes were reported to be 5.6/5.5; 23/22; 89/79 and 324/346 mg/kg bw per day for males and females in the four treatment groups, respectively.

No deaths occurred during the course of the study. Food consumption was reduced (approximately 25%) in males at the highest dose, although when expressed per kg bw, food consumption was only marginally less than in the controls (–8.4%). Body-weight gain was decreased in females at 8000 ppm (30%), although this might have been linked to a higher mean starting weight. Relative liver weights were increased at 8000 ppm in males (30%) and females (25%) and at 2000 ppm in females (25%). Absolute and relative adrenal weights were increased in a dose related manner (30–60%) in males at 500 ppm and above and in females at 8000 ppm (50%). A slightly irregular heart rhythm was present in one female at 8000 ppm. There were no test substance related changes in gross necropsy and histopathology. The increased liver weights were not considered to be adverse in the absence of any histopathological correlates.

The NOAEL was 125 ppm, equal to 5.5 mg/kg bw per day, on the basis of increased adrenal weights in males (Spicer, 1984a).

Table 3. Selected findings in rats given diets containing hexythiazox for 90 days

Parameter	Dietary concentration (ppm)				
	0	10	70	500	3500
Body weight (g):					
Males	327	324	323	323	315*
Females	187	185	184	181*	172***
Body-weight gain (g):					
Males	190	187	186	186	178*
Females	82	81	79	76**	68***
Cholesterol (mg %):					
Males	22	20	21	24	30***
Females	33	32	31	32	41***
Total protein (g %):					
Males	5.9	5.8	5.9	6.0	6.6***
Females	6.0	5.9	5.9	6.1	6.6***
Albumin (g %):					
Males	3.8	3.8	3.8	3.9*	4.2***
Females	3.9	3.8	3.8	3.9	4.2***
Calcium (mg %):					
Males	9.8	9.8	9.9	9.9	10.1**
Females	9.5	9.4	9.5	9.6	9.8**
Alkaline phosphatase (mU/ml):					
Females	88	86	85	85	71***
> 300 mg protein/dl urine:					
Males	0	0	0	0	4
Erythrocytes (10^6/mm^3):					
Males	8.27	8.28	8.19	8.17	7.93**
Packed cell volume[a] (%):					
Males	51.0	51.1	50.7	50.4	48.4**
Haemoglobin (g/dl):					
Males	16.8	16.8	16.8	16.6	16.0**
MCV:					
Males	61.6	61.7	61.9	61.7	61.0*
Platelets (10^6/mm^3):					
Males	0.618	0.641	0.641	0.630	0.650*
Leukocytes (10^3/mm^3)					
Males, month 1.5	8.0	8.0	7.8	7.2*	7.0*
Males, month 3	7.5	7.2	6.9	6.9	7.0

From Takaori et al. (1983b)

MCV, mean corpuscular volume.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student t-test)

[a] Packed cell volume is equivalent to erythrocyte volume fraction × 100.

Table 4. Organ weights in rats given diets containing hexythiazox for 90 days

Parameter	Dietary concentration (ppm)				
	0	10	70	500	3500
Body weight (g):					
Males	327	324	323	323	315*
Females	187	185	184	181*	172***
Absolute liver weight (g):					
Males	8.21	8.22	8.13	8.77**	11.53***
Females	4.40	4.46	4.45	4.79***	6.11***
Relative liver weight:					
Males	2.64	2.66	2.69	2.85***	3.82***
Females	2.52	2.59	2.60*	2.85***	3.82***
Absolute spleen weight (g):					
Males	0.631	0.643	0.626	0.609	0.580***
Females	0.437	0.435	0.430	0.419	0.363***
Relative spleen weight:					
Males	0.203	0.209	0.207	0.199	0.192**
Females	0.250	0.252	0.251	0.250	0.228***
Absolute adrenal weight (g)					
Males (right/left)	0.025/0.026	0.026/0.029	0.026/0.028	0.026/0.028	0.029***/0.032*
Females (right/left)	0.029/.032	0.030/0.031	0.028/0.030	0.028/0.031	0.028/0.031
Absolute kidney weight (g)					
Males (right/left)	1.06/1.07	1.07/1.07	1.05/1.05	1.07/1.06	1.08/1.10
Females (right/left)	0.67/0.66	0.68/0.68	0.67/0.67	0.67/0.68	0.66/0.67
Relative kidney weight					
Males (right/left)	0.34/0.34	0.35/0.35	0.35/0.35	0.35/0.35	0.36**/0.36**
Females (right/left)	0.39/0.38	0.39/0.39	0.39/0.39	0.40/0.40*	0.41***/0.42***
Relative testis weight (right/left)	0.46/0.48	0.47/0.48	0.48/0.46	0.47/0.49	0.48/0.51**
Relative ovary weight (right/left)	0.018/0.020	0.022/0.020	0.20/0.21	0.021*/0.022*	0.023***/0.022*

From Takaori et al. (1983b)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student t-test)

Groups of four male and four female beagle dogs were given diets containing hexythiazox (purity, 97.7%) at a concentration of 100, 500 or 5000 ppm (corresponding to mean daily intakes of 2.9/3.2; 13.1/13.9; 153/148 mg/kg bw per day in males and females, respectively) for 1 year. Body weights and food consumption were recorded weekly. Dogs were observed for moribundity and mortality and overt toxicity twice per day throughout the study; detailed observations were conducted at least once a week. Ophthalmoscopic examinations were conducted pre-test and at weeks 27 and 51. Blood biochemical, haematological and urine-analysis parameters were determined before study initiation and at 3, 6 and 12 months. Cholinesterase activities of erythrocytes and serum were determined. Gross necropsy and histopathology of an extensive range of organs and tissues were performed on all dogs.

The homogeneity and content of the diet were confirmed analytically.

All dogs survived to study termination. An increase in salivation (ptyalism) was reported in some dogs during the later stages of the study, although there was no clear dose–response relationship. There were no statistically significant differences in mean body weight values in any treated group when compared with the control group. However, body-weight gain was decreased in males at the highest dose and all treated females when compared with controls. Food consumption was decreased in all treated groups generally throughout the study (Table 7), but without any clear dose–response relationship.

Values for erythrocytes, haemoglobin and haematocrit (erythrocyte volume fraction) were reduced statistically significantly in males at 500 and 5000 ppm males at 3 months (Table 5); reticulocyte numbers were similar in test and control groups. Serum alkaline phosphatase (significant in males and females) and alanine aminotransferase (significant only in females) activities were increased in males and females (Table 5). There were significant reductions in total protein at 12 months at 5000 ppm in males and females and at 500 ppm in males. Phosphorus was significantly decreased in females at 500 and 5000 ppm and non-significantly in males at 5000 ppm at study termination (Table 5). There were no changes in serum or erythrocyte cholinesterase activities.

Absolute adrenal and adrenal/body weights were significantly ($p < 0.01$) increased at 5000 ppm in males and females (Table 7). This finding was attributed to adrenocortical hypertrophy seen in all three zones of the cortex (Table 6). Increases in adrenal weights were apparent at 500 ppm, the values were not statistically significant but there were trace levels of adrenal hypertrophy (Table 6). Liver/body weight was significantly ($p < 0.05$) increased in males and non-significantly in females at 5000 ppm (Table 7). Liver-weight increase was related to hepatocellular hypertrophy of generally mild severity (Table 6) and serum-enzyme changes (Table 5). Thyroid and parathyroid weights were increased in males at 5000 ppm and in females at 500 and 5000 ppm, but the differences from control values were not statistically significant and there were no consistent histopathological findings. There were no test substance-related changes in urine analysis or ophthalmoscopy.

The NOAEL was 100 ppm, equal to 2.9 mg/kg bw per day, on the basis of increased adrenal weights and adrenal hypertrophy in males and females and indications of altered erythrocyte parameters in males at 500 ppm (Spicer, 1984b).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 80 male and 80 female B6C3F$_1$ mice were given diets containing hexythiazox (purity, 98.2%) at a concentration of 0, 40, 250 or 1500 ppm for up to 2 years (104 weeks). Fifty males and 50 females per group were exposed for 104 weeks, while satellite groups of 10 males and 10 females were scheduled for interim termination at weeks 26, 52 and 78. Body weight and food consumption were determined regularly. Haematological, clinical chemistry and urine analysis examinations were performed on 8–10 males and 8–10 females of each group at weeks 26, 52, 78 and 104. A gross examination was performed on all rats at termination, when the weights of a range of organs were determined. Histopathology was performed on an extensive range of tissues from 10 males and 10 females per group killed at week 52 weeks and at week 104 on all mice that survived until study termination, as well as on all mice that died or were killed in a moribund condition during the study. Livers from the mice killed at week 78 were investigated microscopically and the results presented in a separate report (Anonymous, 1985)

Table 5. Selected haematological and biochemical parameters in a 1-year study in dogs given diets containing hexythiazox

Parameter	Month	Dietary concentration (ppm)			
		0	100	500	5000
Males (n = 4)					
Erythrocytes (×°10^6/mm^3)	0	6.05	6.05	5.71	6.18
	3	6.81	6.55	6.27*	6.16**
	12	6.97	6.99	6.40	6.25
Haemoglobin (g/dl)	0	15.6	15.8	14.8	15.9
	3	18.4	17.5	16.9*	16.7*
	12	18.4	18.4	17.4	17.1
Haematocrit[a] (%)	0	43.9	43.7	41.3	44.2
	3	49.8	48.1	46.1*	45.4**
	12	50.7	51.1	47.3	46.6
Alkaline phosphatase (IU/l)	0	76	72	70	71
	3	46	47	48	81
	12	40	39	41	108**
Alanine aminotransferase (IU/l)	0	31	33	32	35
	3	45	43	41	60
	12	37	41	29	47
Total protein (g/dl)	0	5.6	5.7	5.6	5.8
	3	6.3	5.9	5.9	5.8
	12	6.9	6.4	5.9*	5.9*
Phosphorus (mg/dl)	0	7.2	7.6	7.7	7.4
	3	4.9	5.3	5.2	4.6
	12	4.0	4.1	4.1	3.4
Females (n = 4)					
Alkaline phosphatase (IU/l)	0	81	54*	68	64
	3	57	31*	43	104**
	12	58	25**	32*	106**
Alanine aminotransferase (IU/l)	0	37	35	34	36
	3	43	41	43	57
	12	27	28	31	40**
Total protein (g/dl)	0	5.3	5.3	5.3	5.5
	3	6.0	5.6	5.9	5.7
	12	6.6	6.0	6.0	5.6*
Phosphorus (mg/dl)	0	6.9	7.1	7.0	7.4
	3	4.6	4.9	4.6	4.8
	12	4.2	4.1	3.7*	3.6*

From Spicer (1984b)

* $p < 0.05$, ** $p < 0.01$ (t-test; Dunnet test)

[a] Haematocrit is equivalent to erythrocyte volume fraction × 100.

Table 6. Histopathology findings in a 1-year study in dogs given diets containing hexythiazox

Observation [a]	Dietary concentration (ppm)			
	0	0	0	0
Adrenal cortex (males / females):				
Trace hypertrophy	0/0	0/0	4/4	0/0
Mild hypertrophy	0/0	0/0	0/0	4/4
Liver (males/females):				
Trace hypertrophy	0/0	0/0	0/0	0/1
Mild hypertrophy	0/0	0/0	0/0	4/3
Thyroid (males/females):				
Parafollicular cell hyperplasia, mild	1/3	3/1	1/0	3/2

From Spicer (1984b)

[a] Four males and four females at each dose.

Diet content and homogeneity were confirmed analytically. Mean consumption of the test substance was 6.7/8.4, 42/51 and 267/318 mg/kg bw per day for males and females in the three treatment groups, respectively.

Survival in all groups was > 90% at week 78 and > 70% at week 104. There was no treatment-related alteration in survival rates or in the incidence of clinical findings in the control and treated groups. An apparent decrease in body weight in all groups of males receiving hexythiazoz could have been due to the unusually high body weight of the controls, particularly in the second half of the study (Table 8); this argument would not apply to the group at the highest dose as there were still notable (> 10%) deficits compared with the values for historical controls. The reductions in body weights in the groups at 40 and 250 ppm relative to those of the concurrent controls were most marked towards the end of the study, when the mice had started to lose body weight; the body weight values for the groups at 40 and 250 ppm were considered to be consistent with those of the historical controls. Body weights of females receiving hexythiazoz were comparable to those of females in the control groups. There were sporadic fluctuations in food consumption and food conversion efficiency, but these were not considered to be an effect of hexythiazox.

A range of changes in erythrocyte parameters were seen at 1500 ppm (Table 9); some were also seen at lower doses, but were not consistently related to duration of dosing (Table 9). Platelet counts were increased in males and females at 1500 ppm at week 52 and in males at 1500 ppm at week 104. Reticulocyte counts were increased at week 26 in all males receiving hexythiazoz and in females at 1500 ppm, and at week 52 in males at 1500 ppm (Table 9), but subsequently were similar to control values. There was a consistent, statistically significant reduction in leukocyte counts in males at 250 and 1500 ppm.

A number of clinical chemistry parameters were altered at 1500 ppm. Minor changes in clinical chemistry findings at 250 ppm (increased phosphate concentration and reduced uric acid) were not considered to be adverse as they were small in magnitude and/or not consistent over time (Table 9). There were no significant findings in the results of urine analysis.

Absolute liver and/or liver/body weights were increased in males and females at 1500 ppm throughout the study (Table 10). Increases in relative testes, brain and kidney weights were considered to be secondary to reduced body weights because there was no increase in absolute organ weights. Absolute adrenal and adrenal/body weights of males at 1500 ppm were increased at week 104, as were adrenal/body weights at week 52 (Table 10).

Table 7. Body, adrenal, liver and thyroid weights and food consumption data a 1-year study in dogs given diets containing hexythiazox

Parameter[a]	Dietary concentration (ppm)			
	0	0	0	0
Males (n = 4)				
Body weight (kg):				
Month 1	10.3	10.3	11.0	10.6
Month 3	12.3	12.2	13.5	11.1
Month 12	12.7	13.0	13.6	11.6
Food consumption (g/day):				
Month 1	309	349	337	315
Month 3	405	366	357	346
Month 12	414	322	309	264*
Females (n = 4)				
Body weight (kg):				
Month 1	8.5	8.1	8.6	8.9
Month 3	10.0	9.3	10.0	10.6
Month 12	11.3	10.1	10.4	10.9
Food consumption (g/day):				
Month 1	288	255	281	384
Month 3	410	328	286	331
Month 12	334	260	270	300
Organ weights				
Absolute adrenal weight (g):				
Males	1.10	1.16	1.41	1.76**
Females	1.28	1.19	1.59	2.26**
Absolute liver weight (g):				
Males	333	304	360	386
Females	308	249	271	353
Relative liver weight (to body weight):				
Males	2.71	2.44	2.82	3.51*
Females	3.03	2.60	2.72	3.35
Absolute thyroid/parathyroid weight (g):				
Males	1.21	0.96	1.38	1.58
Females	0.97	0.85	1.29	1.46
Relative thyroid/parathyroid weight (to body weight):				
Males	10.09	7.83	10.39	14.30
Females	9.36	8.67	12.80	14.00

From Spicer (1984b)

Table 8. Mean body weights (g) in male mice given diets containing hexythiazox for up to 2 years and in male mice in the database of historical controls

Time-point	Mean body weight (g)				
	Historical control groups[a]	Dietary concentration (ppm)			
		0 (concurrent control group)	40	250	1500
Week 0	21.3	22.1	22.2	22.1	22.0
Week 52	45.2	47.1	45.4	43.9**	42.7**
Week 78	45.4	48.2	45.7	44.0**	42.9**
Week 86	NA	47.9	44.9	44.5**	42.3**
Week 104	42.7	46.0	41.0**	42.3**	37.8**

From Inoue (1985)

NA, not available.

** $p < 0.01$ versus concurrent controls (group 0).

[a] Nine studies with 650 mice at initiation.

Table 9. Haematological and clinical chemistry parameters in mice given diets containing hexythiazox for up to 2 years

Parameter	Week	Dietary concentration (ppm)			
		0	40	250	1500
Males					
Erythrocytes (10^6/mm^3	26	8.94	8.99	8.82	8.66**
	52	8.87	8.45**	8.43**	8.24***
	78	8.80	8.66	8.33	8.01**
	104	10.35	9.07	9.03	9.47
Haematocrit[a] (%)	26	42.3	42.1	41.3	41.1*
	52	40.8	39.1*	39.1*	38.6**
	78	39.3	38.5	37.9	37.2*
Reticulocytes (%)	26	15	19*	21**	21*
	52	11	15	14	16*
Leukocytes (10^3/mm^3)	26	3.0	2.6	1.8*	1.2**
	78	3.0	2.5	1.7**	1.6**
	104	4.2	2.6	2.1*	1.6**
Uric acid (mg/dl)	52	3.7	4.2	3.7	2.4***
	78	4.3	4.2	3.9	3.7
Females					
Erythrocytes (10^6/mm^3)	26	9.31	9.34	9.15	8.91***
	52	8.82	8.86	8.65	8.74
	78	8.77	8.80	8.72	8.41*

Parameter	Week	Dietary concentration (ppm)			
		0	40	250	1500
	104	8.84	8.77	8.54	8.23
Haematocrit (%)[a]	26	43.4	43.3	43.0	42.0**
Reticulocytes (%)	26	17	19	17	24*
Uric acid (mg/dl)	52	3.2	2.6	2.1**	2.2**
	78	3.9	4.5	4.4	3.0***
Phosphate (mg/dl)	52	6.0	5.6	6.1	6.1
	78	4.4	5.0	5.2**	5.9***
	104	6.0	6.1	6.3	6.4

From Inoue (1985)

[a] Haematocrit is equivalent to erythrocyte volume fraction × 100.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 10. Mean organ weights in mice given diets containing hexythiazox for up to 2 years

Parameter	Week	Dietary concentration (ppm)			
		0	40	250	1500
Absolute liver weight (g), males/females:	26	1.25 / 1.10	1.21 / 1.09	1.31 / 1.12	1.43** / 1.26*
	104	1.95 / 1.46	2.02 / 1.61	1.87 / 1.51	2.59* / 1.69*
Absolute adrenal weight (mg), males	52	4	4	4	6
	104	6	6	7	7*

From Inoue (1985)

* $p < 0.05$ relative to controls; ** $p < 0.01$ relative to controls.

An increased incidence of liver nodules in males and females at 1500 ppm was observed on gross pathological examination. Cytological alterations in liver were increased in treated males at interim kill at week 52. Liver necrosis was increased in males at 1500 ppm at study termination (Table 11) but not in mice dying or killed earlier in the study. Changes in ovarian atrophy and hyaline droplet formation in the central nervous system were not considered to be adverse effects as there was no clear dose–response relationship and these effects are known to be age-related; it is also of note that the mean ovarian weights in mice in the control group were very high and showed a large standard deviation. Proteinaceous casts in the kidney were increased in frequency in males and females at 250 and 1500 ppm at study termination, but with no clear dose–response relationship (Table 11). Adrenal findings were similar in groups receiving hexythiazoz and in the control groups. The incidences of hepatocellular adenoma and carcinoma were increased in males at 1500 ppm, but not statistically significantly ($p > 0.05$). In females, the incidence of hepatocellular adenoma was increased significantly ($p = 0.033$) at 1500 ppm, but there was no change in the incidence of hepatocellular carcinoma in females. Low incidences of hepatoblastoma were seen in 3 out of 70 males at 1500 ppm, compared with a mean incidence in historical controls of 0.2% (range, 0 out of 50 to 1 out of 50; National Toxicology Program, 1999). Two of the three mice with hepatoblastoma also had

hepatocellular adenoma and carcinoma, and the hepatoblastomas were considered to be part of the general pattern of liver tumours in these aged mice. The incidences of hepatocellular adenomas and carcinomas were related to age and/or extended duration of treatment as they were not increased in mice killed or dying before week 78, the normal duration of a study of carcinogenicity in mice. The histopathology findings, particularly the liver, were re-evaluated and although absolute numbers of mice with a particular lesion varied between the reports the overall picture was consistent (Enomoto, 1986; Inoue & Enomoto, 1987a & 1987b).

Table 11. Re-evaluation of incidence of non-neoplastic and neoplastic histopathology findings in mice given diets containing hexythiazox for up to 2 years

Finding	Dietary concentration (ppm)				No. of mice examined
	0	40	250	1500	
Males					
Liver:					
Hepatic nodules:					
Week 104	15	9	16	35***	70
Hepatocellular adenoma:					
Week 78	9	3	3	2	70
Week 104	20	23	17	27	
Hepatocellular carcinoma:					
Week 78	0	1	1	0	70
Week 104	11	9	10	14	
Hepatoblastoma: week 104	0	0	0	3	70
Hepatic necrosis:					
Dying during study	4	4	3	1	70
Terminal sacrifice	0	1	3	7*	
Kidney:					
Proteinaceous casts: week 104	12	9	20*	19	60
Females					
Liver:					
Hepatic nodules	6	6	3	19**	70
Hepatocellular adenoma:					
Week 78	2	0	0	0	70
Week 104	7	1*	5	16*	
Hepatocellular:					
Week 78	0	0	0	0	70
Week 104	0	3	3	3	
Hepatoblastoma: week 104	0	0	0	1	70
Kidney:					
Proteinaceous: week 104	24	29	37*	32	60
Ovarian atrophy: week 104	31	43	47	43	60

From Inoue (1985); re-evaluated by Enemoto (1986); Inoue & Enomoto (1987a & 1987b)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Fisher exact test).

The NOAEL for toxicity was 40 ppm, equal to 6.7 mg/kg bw per day, on the basis of consistently reduced leukocyte counts in males and increased proteinaceous casts in the kidney of males and females at 250 ppm. The NOAEL for carcinogenicity was 250 ppm, equal to 42 mg/kg bw per day, on the basis of the increased incidence of hepatocellular adenoma in females at 1500 ppm (Inoue, 1985).

Rats

Groups of 80 male and 80 female Fischer F344 rats were given diets containing hexythiazox (purity, 98.2%) at a concentration of 0, 60, 430 or 3000 ppm for up to 2 years. Groups of 10 males and 10 females per dose were selected from a satellite group of 30 males and 30 females designated for water consumption measurements, clinical pathology and interim necropsy. Ophthalmoscopic examinations were conducted on all rats pre-test and on all rats in the control group and the group at the highest dose (excluding those in the satellite groups) at weeks 6, 13, 25, 52, 78 and 104. Haematological and clinical chemistry measurements and urine-analysis determinations were performed on 10 males and females per group at weeks 26, 52, 78 and 104. Necropsy was performed on all rats that died spontaneously or were killed in extremis, on rats killed for interim necropsy and on all survivors at week 104. Organ weights for adrenals, brain (with stem), heart, kidneys, liver, lung, ovaries, spleen and testes were determined. Samples of a range of tissues were collected from all rats for microscopic examination. In addition, three transverse sections through the head, including tongue, nasal cavity, turbinates, paranasal sinuses, nasopharynx, portions of oral cavity and middle ear, were examined for 10 males and 10 females per group. If tumours occurred at one of these sites, sections from that site were examined microscopically for all rats.

The homogeneity and content of the diet were confirmed analytically. Mean intakes of test substance were 3.2/4.0, 23/29 and 163/207 mg/kg bw per day for males and females in the three treatment groups, respectively.

There were no major differences in mortality among the groups; overall survival was good at > 70% in all groups at week 104. Mean body weights were statistically significantly lower throughout the study at 3000 ppm when compared with those of the controls. At 430 and 60 ppm, body weights were slightly decreased in males and females, but the magnitude was < 5% and although the difference compared with controls was occasionally statistically significant, it was not considered to be adverse. Food consumption was increased approximately 10% in males and females at 3000 ppm on a number of occasions. Food efficiency showed great variability throughout the study. Water consumption was generally comparable in controls and treatment groups. The only finding of note on clinical examination was an increase in swollen/withdrawn testes from month 15 months at 430 and 3000 ppm (Table 12). There were no treatment-related adverse findings in haematological, clinical chemistry or urine-analysis examinations. No test substance -elated ophthalmoscopic findings were observed.

Absolute and relative liver weights were increased in males and females at 3000 ppm at study termination. Absolute and relative adrenal weights were increased in males at 60 and 3000 ppm at study termination, but not at 430 ppm; with no dose–response relationship, the finding at 60 ppm was considered to be incidental. Relative kidney weights were increased significantly at 3000 ppm at study termination; absolute kidney weights were increased significantly in females, and increased but not statistically significantly in males (Table 13). Absolute and relative spleen weights were decreased (10–15%) in males at 3000 ppm at 12 months, but increased at 24 months (Table 13). Increased absolute and relative testis weights were observed at 3000 ppm at interim kill and increased testis/body weight at study termination. Relative ovary weights were increased significantly with a non-significant increase in absolute ovary weight at 3000 ppm (Table 13). Relative weights of the brain, heart and lung were increased at 3000 ppm, but as there was no associated increase in absolute weights these were considered to be secondary to the lower body weight.

Table 12. Clinical findings in the testes of rats (n = 70) given diets containing hexythiazox for up to 2 years

Finding	Dietary concentration (ppm)			
	0	60	430	3000
Testes withdrawn or swollen				
Week 66–78	5	2	6	26*
Week 79–91	23	19	43*	51*
Week 92–106	31	29	45*	45*

From Spicer (1984c)

* $p < 0.05$

Table 13. Body and organ weights in rats given diets containing hexythiazoz for up to 2 years

Body or organ	Dietary concentration (ppm)			
	0	60	430	3000
Males				
Body weight (g)	378	374	362*	343*
Absolute adrenal weight (g)	64	70*	64	68*
Relative adrenal weight [a] (% × 10^3)	17	19*	18	20*
Absolute kidney weight (g)	3.6	3.5	3.5	3.6
Relative kidney weight [a] (% × 10)	1.0	0.9	1.0	1.1**
Absolute spleen weight (g)	1.3	1.3	1.4	1.7*
Relative spleen weight [a] (%)	0.33	0.35	0.40*	0.50*
Absolute liver weight (g)	15.7	15.6	15.8	18.1**
Relative liver weight [a] (%)	4.2	4.2	4.4	5.3**
Absolute testis weight (g)	5.9	5.5	5.9	6.6
Relative testis weight [a] (%)	1.6	1.5	1.6	1.9**
Females				
Body weight (g)	279	279	272	247**
Absolute adrenal weight (mg)	75	68**	70*	73
Relative adrenal weight [a] (% × 10^3)	27	25**	26	30**
Absolute kidney weight (g)	2.6	2.6	2.7	2.8**
Relative kidney weight [a] (%)	0.9	0.9	1.0	1.1**
Absolute spleen weight (g)	0.8	0.9	0.9	0.8
Relative spleen weight (%)	0.30	0.33	0.35	0.32
Absolute liver weight (g)	11.9	11.9	11.8	13.1**
Relative liver weight [a] (%)	4.3	4.3	4.4	5.4**
Absolute ovary weight (mg)	128	122	130	134
Relative ovary weight (% × 10^2)	4.6	4.4	4.8	5.4**

From Spicer (1984c)

[a] Compared with body weight.

* $p < 0.05$; ** $p < 0.01$

The incidences and severity of vacuolar fatty changes in the adrenal were increased in females at 430 and 3000 ppm at termination and in males at 3000 ppm at interim and terminal kill (Tables 14 and 15). Chronic nephritis was a very common finding both in the control group and in rats receiving hexythiazoz, but the severity was increased in rats treated with hexythiazoz, most notably at interim kill, at dietary concentrations of 430 ppm and greater, although statistical significance was apparent only in males at the highest dose (Table 14). Cytoplasmic alteration in males at 3000 ppm was the only histopathological finding in the liver that was increased when compared with incidence in rats in the control group. Seminal tubule vesiculitis was increased in males at 3000 ppm. Changes in thyroid histopathology were not consistent in males and females and did not attain statistical significance. Reduced incidences of eye mineralization and of heart muscle degeneration were seen in males and females at 3000 ppm. Other findings were typical of aged F344 rats and were similar in control and treated groups. The incidence of mammary-gland fibroadenoma was increased in males at 3000 ppm compared with values for historical controls (0–6%; Anonymous, 2007). The incidence of testicular interstitial-cell adenoma was increased at 3000 ppm at the interim 12-month kill (Table 14) relative to values for historical controls (0–15%); the incidence in rats in the control group at study termination was > 90%, as is typical for the F344 strain (Table 15). The size of interstitial-cell tumours and the occurrence of withdrawn/swollen testes might be related, but no specific measurements of tumour size were reported. Overall rates of tumour incidence (benign and malignant) were similar in rats receiving hexythiazoz and in the control group.

The NOAEL for general toxicity was 60 ppm, equal to 3.2 mg/kg bw per day, on the basis of increases in fatty vacuolation of the adrenals in males and females and the severity of chronic nephritis and incidences of swollen/withdrawn testes in males. The NOAEL for carcinogenicity was 430 ppm, equal to 23 mg/kg bw per day, on the basis of increases in mammary-gland fibroadenomas in males at 24 months (2 years) and in testicular interstitial-cell adenomas at 12 months relative to historical control incidences (Spicer 1984c).

Table 14. Histopathology findings at 12-month interim kill in male rats given diets containing hexythiazox

Finding	Dietary concentration (ppm)			
	0	60	430	3000
Adrenal gland:				
Fatty vacuolation	2/10	1/10	5/10	9/11*
Kidney:				
Chronic nephritis (total)	7/10	6/10	9/10	8/11
Chronic nephritis, > trace	1/10	1/10	4/10	6/11*
Testes:				
Interstitial-cell adenoma	0/10	0/10	2/10	3/11 [a]
Interstitial-cell hyperplasia [b]	4/10	3/10	3/10	6/10

From Spicer (1984c)

* $p < 0.05$, Fisher exact test.

[a] $p = 0.0245$ (chi-squared test for linear trend).

[b] No hypertrophy reported in any rat.

Table 15. Histopathology findings in rats (n = 70) given diets containing hexythiazox for 2 years

Finding	Dietary concentration (ppm)			
	0	60	430	3000
Males				
Adrenal, cortex:				
Fatty vacuolation, total	13	12	17	24*
Fatty vacuolation, > mild	10	11	15	15
Adrenal, medulla:				
Pheochromocytoma	5	9	9	7
Pheochromocytoma, malignant	0	0	0	1
Kidney:				
Chronic nephritis, total	68	66	70	69
Chronic nephritis, mild	35	18	28	18
Chronic nephritis, moderate	28	46	34	50*
Chronic nephritis, severe	2	0	0	0
Liver:				
Cytoplasmic alteration, clear	3	4	4	7
Cytoplasmic alteration, > trace	1	4	4	6
Mammary gland:				
Fibroadenoma	0	1	2	6
Adenocarcinoma	0	0	0	1
Seminal vesicle:				
Vesiculitis, total	5	2	6	12
Testis:				
Interstitial-cell adenoma	67	66	66	68
Thyroid:				
Parafollicular cell adenoma	3	3	2	7
Follicular carcinoma	0	0	1	1
Females				
Adrenal, cortex				
Vacuolar change, total	18	20	30*	28
Vacuolar change , ≤ mild	17	18	26	28*
Adrenal, medulla				
Pheochromocytoma	0	3	0	2
Pheochromocytoma, malignant	0	0	0	1
Kidney:				
Chronic nephritis, total	61	60	67	66
Chronic nephritis, mild	28	36	48	44
Chronic nephritis, moderate	11	10	5	14
Chronic nephritis, severe	0	1	0	0
Thyroid:				
Parafollicular cell hyperplasia	4	8	7	7
Parafollicular cell adenoma	3	3	3	3

From Spicer (1984c)

* $p < 0.05$

2.4 Genotoxicity

Hexythiazox has been tested for genotoxicity in a wide range of assays. Many of the studies were performed in the 1980s and did not comply with GLP or current test guidelines. However, the Meeting considered that the overall database was adequate to conclude that hexythiazox does not possess significant genotoxic potential. The results of Ames tests with *Salmonella typhimurium* and *Escherichia coli* were negative. The result of a test for mutation in *Saccharomyces cerevisiae* was considered equivocal in the presence of metabolic activation. A test for mutation at the *Hprt* gene locus in Chinese hamster V79 cells gave negative results. The results of an assay for bacterial recombination (supplemental study) and a test for unscheduled DNA synthesis in rat primary hepatocytes in vitro were negative. The result of an adequate test for chromosomal aberration in vitro in Chinese hamster ovary (CHO) cells was negative. An inadequate test for chromosomal aberration in vivo gave negative results, as did two assays for micronucleus formation in vivo. The weight of evidence is that hexythiazox has no significant genotoxic potential.

The results of studies of genotoxicity with hexythiazox are summarized in Table 16.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 30 male and 30 female Wistar rats (age 5 weeks) were given diets containing hexythiazox (purity, 98.2%) at a concentration of 0, 60, 400 or 2400 ppm. There were two litters per generation. F_0 parents received treated diets for 13 weeks before the F_1a mating; the F_1b mating occurred 2 weeks after weaning. F_1b parents were first mated at age 17 weeks. All pups were observed until day 21 of lactation. Before matings, the estrus status of females was confirmed. Litters were culled by random selection to a total of eight pups (four males and four females) on postnatal day 4. Mortality, behaviour, clinical observations, body weights, food consumption, mating performance, duration of gestation, reproduction and litter data were examined. F_1a and F_2a pups were observed for mortality only. F_1b and F_2b offspring were examined for general condition and physical development (pinna-detachment, eruption of incisors, hair growth and eye-opening). Brain, heart, lungs, liver, kidneys, spleen, adrenals, testes and ovaries weights were recorded from 10 F_2 adult males and 10 adult females per dose; for F_0 and F_1 rats, tissues were taken from five males and five females per group. A wide range of tissues was collected for histopathological examination from these rats, but epididymides, seminal vesicles and coagulating glands were not preserved for histopathology. The study also included a satellite group subjected to limited investigation of developmental effects in five F_0 dams per dose.

The homogeneity and content of the diets were confirmed analytically. Achieved intakes were 3.6–9.2, 24–62 and 136–360 mg/kg bw per day at 60, 400 and 2400 ppm respectively.

One death was observed at 60 ppm in the F_0 generation. Pup mortality did not show dose-related effects. There were no remarkable clinical signs of toxicity. Mean body weight was significantly reduced in F_0, F_1 and F_2 males and females at 2400 ppm. Food consumption was reduced in the F_0 and F_2 generations, and in F_1 females at 2400 ppm and also at 400 ppm in the F_2 females. The food efficiency of F_0 and F_1 males at 2400 ppm tended to be lower than that in the control group. There were significant increases in organ weights at 2400 ppm, but no dose-related histopathological findings. Absolute and relative liver weights were increased in males and females at the highest dose in all generations.

There were no adverse effects on mating, pregnancy or litter parameters at any mating (Table 17). There were no adverse findings in the satellite group for investigation of developmental effects.

Table 16. Results of studies of genotoxicity with hexythiazox

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	100, 200, 400, 800, 1600, 3200 and 6400 µg/plate, ± S9, in DMSO	97.7	Negative ± S9	Inoue (1983a)
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* (WP2 *uvr*A)	313, 625, 1250, 2500 and 5000 µg/plate, ± S9, in DMSO	99.9	Negative ± S9	Kanaguchi (2005)
Gene mutation	*Saccharomyces cerevisiae* S138, S211 and D_4	S138 and S211: 312, 625, 1250, 2500, 5000 and 10 000 µg/ml	98.4	Negative ± S9	Jagannath (1984)
		D_4: 1, 10, 100, 500, 1000, 2500, 5000 and 10 000 µg/plate		Equivocal + S9; negative – S9	
		Both tests ± S9, in DMSO			
Gene mutation (*Hprt*)	Chinese hamster ovary cells (V79)	9.38, 18.8, 37.5, 75.0 and 150 µg/ml [a] ± S9; in DMSO	98.6	Negative ± S9	Seeberg (1986)
Chromosomal aberration	Chinese hamster ovary (CHO) cells	20, 30, 40 and 50 µg/ml [a] – S9, in DMSO;	98.4	Negative ± S9	Galloway (1984)
		40, 100, 150, 200, 300 and 400 µg/ml [a] + S9, in DMSO			
Chromosomal aberration	Chinese hamster ovary (CHO) cells	5, 20, 35 and 50[b] µg/ml – S9 in DMSO;	Not reported	Negative ± S9	Ivett (1986)
		35, 50, 200, 350 and 500[b] µg/ml + S9, in DMSO			
Recombination	*Bacillus subtilis* H17 and M45	400, 800, 1600 and 3200 µg/disk, in DMSO	97.7	Negative	Inoue (1983b)
Unscheduled DNA synthesis	Rat primary hepatocytes	2.5, 5, 10, 25, 50,[b] 100 and 250 µg/ml	98.4	Negative	Cifone (1985)
In vivo					
Chromosomal aberration	Chinese hamster bone marrow	1000, 2000 and 4000 mg/kg bw, in peanut oil and DMSO	98.6	Negative, but several shortcomings	Mosesso (1986)
Micronucleus formation	CD-1 mouse bone marrow	100, 500 and 1000 mg/kg bw, in DMSO	98.4	Negative	Ivett (1984)
Micronucleus formation	ICR mouse bone marrow	500, 1000 and 2000 mg/kg bw, in corn oil	99.0	Negative	Gudi & Krsmanovic (2001)

S9, 9000 × *g* supernatant from rat livers.

[a] Precipitation and cytotoxicity at the highest dose.

[b] Cytotoxicity.

The mean body weights of male and female F_1b and F_2b pups decreased at 2400 ppm and slightly at 400 ppm in F_2b females. The eruption of incisors was promoted in F_1b pups and the hair growth of the abdomen was delayed in all treated F_1b pups and in F_2b pups at 2400 ppm. In addition, eye opening was delayed at 2400 ppm in the F_2b pups. The delays in developmental markers were probably secondary to the lower body-weight gain, in addition, the alterations in developmental markers were not consistent in the two generations.

Table 17. Litter data and body weights of pups during lactation in a multigeneration study in rats given diets containing hexythiazox

Parameter	Dietary concentration (ppm)			
	0	60	400	2400
Mean live litter size at birth:				
F_1a	10.3	9.5	10.1	9.7
F_1b	7.9	8.5	9.9	10.1
F_2a	8.9	7.8	9.7	7.9
F_2b	8.7	9.1	9.2	9.0
Mean litter size at day 21:				
F_1a	7.7	7.1	7.7	7.4
F_1b	6.7	6.9	7.3	7.5
F_2a	7.0	6.4	7.4	6.6
F_2b	6.8	6.3	7.4	7.1
Mean pup weight (g) on day 0:				
F_1b (male/female)	5.3 / 5.0	5.3 / 5.0	5.2 / 4.8	5.2 / 4.9
F_2b (male/female)	5.3 / 4.9	5.1* / 4.8	5.1 / 4.8	5.3 / 4.9
Mean pup weight (g) on day 4:				
F_1b (male/female)	8.7 / 8.4	8.9 / 8.5	8.5 / 8.1	7.8***/7.5***
F_2b (male/female)	8.0 / 7.6	7.8 / 7.6	7.5 / 7.3	7.2 / 6.8
Mean pup weight (g) on day 7:				
F_1b (male/female)	12.9 / 12.4	13.2 / 12.6	12.7 / 12.0	11.3***/11.1***
F_2b (male/female)	11.7 / 11.2	11.5 / 11.1	11.2 / 10.7	9.9***/9.4***
Mean pup weight (g) on day 28:[a]				
F_1b (male/female)	61.0 -/ 55.2	60.5 / 55.0	60.0 / 54.8	54.8**/50.5**
F_2b (male/female)	55.4 / 50.7	51.0 / 48.4	50.1 / 46.0	45.5*/43.6*

From Okugi & Enomoto (1984)

[a] No. of dams = 5

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

The NOAEL for effects on reproduction was 2400 ppm, equal to 136 mg/kg bw per day, the highest dose tested. The NOAEL for parental toxicity was 400 ppm, equal to 24 mg/kg bw per day, on the basis of decreased body weights and food consumption. The NOAEL for offspring toxicity was 400 ppm, equal to 24 mg/kg bw per day, on the basis of reduced body-weight gain during lactation (Okugi & Enomoto, 1984).

(b) Developmental toxicity

Rats

Groups of 24 mated female Sprague-Dawley (Crj:CD) rats received daily oral doses of hexythiazox (purity, 98.3%) at 0, 240, 720 or 2160 mg/kg bw per day by gavage in 5% aqueous gum arabic from day 7 to day 17 of gestation. The day of copulation was classed as day 0, therefore dosing should have started on day 6. Dams were killed on day 21, all viable fetuses were weighed, sexed and examined for external malformations and variations including the palate and eyes. Approximately one third of each litter was preserved in Bouin fixative and examined for visceral malformations and variations. The remaining two thirds of each litter was fixed in ethanol and cartilage and bone were differentially stained with alcian blue and alizarin red S and examined for skeletal malformations and variations.

Significant reductions in mean maternal body-weight gain were observed at 720 and 2160 mg/kg bw per day during dosing; these were probably secondary to reduced food consumption (Table 18). The changes in mean body weight over the first 2 day of dosing appeared to show a treatment-related effect, but there were no biologically significant differences between the groups when the ranges were taken into account. There was a significant decrease in absolute heart and spleen weights in dams at 720 mg/kg bw per day compared with controls. The relative spleen weight was also reduced at 720 mg/kg bw per day. Absolute and relative ovary weights were increased at 2160 mg/kg bw per day and the relative value was also increased at 720 mg/kg bw per day. There was no significant change in fetal viability, fetal weights, placental weights or any other reported parameters. The number of fetuses with subcutaneous haemorrhages, dilation of the renal pelvis and non-ossified metatarsi were slightly increased, especially at 2160 mg/kg bw per day. Only the increase in non-ossified metatarsi was statistically significant. The incidence of non-ossified metatarsi was also statistically significantly increased at 720 mg/kg bw per day. The observed changes did not indicate any teratogenic potential for hexythiazox.

Table 18. Findings of a study of developmental toxicity in rats given hexythiazox by gavage

Finding	Dose (mg/kg bw per day)			
	0	240	720	2160
Maternal body weight (g), range:				
Day 7 of gestation (min/max)	270 / 324	282 / 334	280 / 321	258 / 334
Day 9 of gestation (min/max)	262 / 331	263 / 337	265 / 321	236 / 338
Body-weight gain (g), range:				
Day 7–9 (min/max)	– 11.1 / + 12.3	– 22.1 / + 12.4	– 27.1 / + 5.2	– 33.4 / + 9.2
Maternal body-weight gain (g):				
Days 0–7 of gestation	30	30	28	30
Days 7–9 of gestation	2.9	– 0.4	– 3.3	– 6.0
Days 7–17 of gestation	55	54	44**	42**
Days 17–21 of gestation	52	57	57	57
Days 0–21 of gestation	136	141	129	129
Food consumption (g/rat per day):				
Days 7–9 of gestation	21.8	20.1	21.8	19.3*
Day 21 of gestation	19	20	19	20
Days 7–17 of gestation	255	250	232	231

Finding	Dose (mg/kg bw per day)			
	0	240	720	2160
Fetal body weights (g) :				
Males / females	5.29 / 5.04	5.26 / 4.98	5.20 / 4.93	5.17 / 4.87
Number of fetuses (litters) with:				
Subcutaneous haemorrhage	1	3	0	6
External malformations	1	1	0	1
Skeletal malformations	0	0	0	1
Visceral variations (dilation of renal pelvis)	7/110	9/118	9/112	11/111
Skeletal variations	24/222	29/240	20/226	24/225
No. of ossified metatarsi	9.9	9.9	9.7**	9.5**
Number of resorptions	15	14	19	15
Mean viable litter size	14.4	15.6	15.4	14.6

From Gotoh et al., (1983)

* $p < 0.05$

The NOAEL for maternal toxicity was 240 mg/kg bw per day on the basis of decreased maternal body-weight gain. The NOAEL for teratogenicity was 2160 mg/kg bw per day, the highest dose tested. The NOAEL for effects on fetal development was 240 mg/kg bw per day on the basis of reduced metatarsal ossification (Gotoh et al., 1983).

Rabbits

Groups of 15 mated female New Zealand White rabbits were given daily oral doses of hexythiazoz (purity, 97.7%) at 120, 360 or 1080 mg/kg bw per day by gavage in 5% arabic gum aqueous solution from day 6 to day 28 of gestation (copulation, day – 1). On day 28 of gestation, the rabbits were killed, the uterus was weighed and the fetuses were removed from the dams by caesarean section. Routine examinations of the uterine contents were performed and all viable fetuses were weighed and examined for external malformations and variations, including the palate and eyes, and fixed in alcohol. Each fetus was dissected, internally sexed and examined for visceral malformations and variations. Skeletal malformations and variations were examined after staining the skeleton with alizarin red S.

Two females died, one in the group at 120 mg/kg bw per day (hind-limb paralysis) and the other at 360 mg/kg bw per day (pulmonary congestion). There were no treatment-related clinical signs or adverse body weight changes during the study (Table 19). Weights of maternal organs and mean fetal body weights were not significantly different between the groups. Incidences of resorptions and malformations were similar in all groups. The number of fetuses with skeletal variations, primarily overlapping of the vertebral arch, was increased at 1080 mg/kg bw per day (Table 19). A later re-analysis (Gotoh & Nishibe, 1988) did not confirm the vertebral arch anomalies and the authors proposed that the these anomalies were an artefact; however, a true artefact would be expected to have a random distribution and not affect only seven animals from the same group.

The NOAEL for teratogenicity and maternal toxicity was 1080 mg/kg bw per day. The NOAEL for fetotoxicity is 360 mg/kg bw per day on the basis of an increased incidence of overlapping of the vertebral arch at the highest dose level, against a zero background rate (Gotoh et al., 1984).

Table 19. Findings of a study of developmental toxicity in rabbits given hexythiazox by gavage

Finding	Dose (mg/kg bw per day)			
	0	120	360	1080
Maternal mean body-weight gains (g):				
Days 0–6 of gestation	0.12	0.16	0.12	0.14
Days 6–18 of gestation	0.10	0.16	0.16	0.13
Days 18–28 of gestation	0.17	0.20	0.28*	0.22
Days 0–28 of gestation	0.39	0.52	0.56*	0.48
Number of resorptions	17	16	12	15
Mean viable litter size	7.0	8.3	8.3	6.8
Mean fetal body weights (g):				
Males / females	35.4 / 35.4	34.2 / 33.7	38.0 / 36.5	34.4 / 34.9
Fetuses/litters with malformations:	3/2	0	4/3	3/3
Cleft palate	0	0	1	0
Absence of tail	0	0	0	1
Cardia bifida	2	0	0	0
Ventricular septal defect	1	0	0	0
Fusion of sternebrae	0	0	1	2
Fusion of ribs	0	0	2	0
Fusion of the thoracic vertebral arches	0	0	1	0
Fetuses with skeletal variations (total):	3 / 98 (3.1%)	2 / 100(2.0%)	2 / 100(2.0%)	12 / 88 (13.6%)
Bilobed shape of the sternebrae	1	0	0	1
Splitting of the sternebrae	1	0	1	2
Bilobed shape of the thoracic vertebral body	1	1	1	1
Asymmetry of the sternebrae	0	1	0	2
Overlapping of the vertebral arch	0	0	0	7
Partial absence of the thoracic vertebral arch	0	0	0	1
Fetuses with visceral variations:	1/98	0	0	2/88

From Gotoh et al. (1984)

2.6 Special studies

(a) Studies on metabolites

Studies of acute toxicity and genotoxicity have been performed on a number of rat metabolites of hexythiazox. The results of these studies are summarized in Tables 20 and 21.

PT-1-2 (5-(4-chlorophenyl)-4-methyl-2-oxo-3-thiazolidine-carboxamide) and PT-1-3 (5-(4-chlorophenyl)-4-methyl-2-oxo-3-thiazolidine) were more acutely toxic orally than hexythiazox. All other metabolites are of relatively low acute oral toxicity and none was genotoxic in Ames tests.

Table 20. Acute toxicity with metabolites of hexythiazox

Test substance[a]	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Purity (%)	Reference
PT-1-2	Rat	SLC:SD	Males	Oral	2321	Approx. 99%	Saika et al. (1985)
			Females		1079		
PT-1-3	Rat	SLC:SD	Males	Oral	494	Approx. 99%	Saika et al. (1985)
			Females		341		
PT-1-4	Rat	SLC:SD	Males & females	Oral	> 5000	Approx. 99%	Saika et al. (1985)
PT-1-5	Rat	SLC:SD	Males & females	Oral	> 5000	Approx. 99%	Saika et al. (1985)
PT-1-6	Rat	SLC:SD	Males & females	Oral	> 5000	Approx. 99%	Saika et al. (1985)
PT-1-8 (*cis*)	Rat	SLC:SD	Males	Oral	> 5000	Approx. 99%	Saika et al. (1985)
PT-1-8 (*trans*)	Rat	SLC:SD	Males & females	Oral	> 5000	Approx. 99%	Saika et al. (1985)
PT-1-9	Rat	SLC:SD	Males & females	Oral	> 5000	Approx. 99%	Saika et al. (1985)

[a] The vehicle for all test substances was water + Tween 80.

[b] No studies of exposure by inhalation were available.

(b) Screening for pharmacological activity

Hexythiazox was extensively investigated, mainly by acute parenteral administration, for pharmacological activity in mice, rats, rabbits and guinea-pigs. In none of the studies did hexythiazox show any indication of potent pharmacological activity.

(i) Effects on the central nervous system

Mice

Mice given hexythiazoz at a dose of 200, 1000 or 5000 mg/kg bw (intraperitoneal, i.p.) showed decreased reactivity/passivity, writhing, piloerection and a decreased heart rate. At 200 mg/kg bw, these signs were very slight and were no longer apparent after 120 min; recovery time was longer at higher doses. Pentobarbital sleeping time was prolonged at 1000 and 5000 mg/kg bw (i.p.) but not at 200 mg/kg bw. There was an anti-convulsive effect (delayed start of convulsions, reduced mortality) against pentetrazole at 45, 60 and 120 mg/kg bw (i.p.), but not at 30 mg/kg bw. There was no effect on strychnine-induced convulsions or mortality at doses of up to 114 mg/kg bw (i.p.), with a weak protective effect at 228 mg/kg bw (mortality, four out of six compared with six out of six in the control group). Locomotor activity in mice was increased at 30 mg/kg bw (intravenous, i.v.); there was no effect at 60 mg/kg bw, and at a dose of 120 mg/kg bw, locomotor activity was reduced.

Rabbits

Hexythiazox did not affect the body temperature of rabbits dosed intravenously at 30 or 60 mg/kg bw. Spontaneous electroencephalogram (EEG) in conscious rabbits was unaffected by dosing with hexythiazox at 50 mg/kg bw.

(ii) Effects on cardiovascular and respiratory system of rabbits

Slight hypotension and hypoventilation lasting for 15 min were observed in rabbits given hexythiazoz intravenously at a dose of 25 mg/kg bw, becoming more pronounced at 50 mg/kg bw. Both doses caused a slight decrease in heart rate.

Table 21. Results of studies of genotoxicity with metabolites of hexythiazox

Test substance (vehicle)	End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro						
PT-1-2	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	0.5–1000 µg/plate + S9 1–5000 µg/plate –S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-3	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	1–5000 µg/plate + S9 5–10 000 µg/plate – S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-4	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	5–10 000 µg/plate ± S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-5	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	10–50 000 µg/plate ± S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-6	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	5–10 000 µg/plate ± S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-8 (*cis*)	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	5–10 000 µg/plate ± S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-8 (*trans*)	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	0.5–5000 µg/plate + S9 5–10 000 µg/plate – S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-9	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	10–50 000 µg/plate ± S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)

S9, 9000 × *g* supernatant from rat livers.

[a] The vehicle for all test substances was dimethyl sulfoxide (DMSO).

(iii) Effects on skeletal and smooth muscle

Rats

Intravenous administration of hexythiazox at doses up to 100 mg/kg bw did not affect rat skeletal muscle tension induced by indirect stimulation. Hexythiazox did not affect the body temperature of rats given intraperitoneal doses of 1250 or 2500 mg/kg bw.

Guinea-pigs

Hexythiazox at concentrations of 0.1 or 1 mg/ml inhibited the contraction of isolated ileum sections induced by acetylcholine; a concentration of 0.01 mg/ml had no effect. The contraction of isolated ileum induced by histamine was inhibited at by hexythiazoz at 1 mg/ml, but not at 0.1 mg/ml. The contraction of the ileum induced by barium chloride was inhibited by hexythiazoz at concentrations of 0.01 mg/ml and above. Hexythiazox at 1 mg/ml slightly inhibited contraction of the vas deferens induced by acetylcholine, but had no effects on contraction induced by adrenaline. Tracheal contractions induced by acetylcholine and relaxation caused by noradrenaline were unaffected by hexathiazox at concentrations of of 0.01 or 0.1 mg/ml. Contractions induced by histamine were slightly inhibited at both of these concentrations.

(iv) Effects on the gastrointestinal tract

Hexythiazox had no effect on intestinal motility in mice when given intravenously at doses of up to 150 mg/kg bw and did not alter gastric secretion in rats given doses of 3000 mg/kg bw by gavage.

(v) Effects on blood

Intraperitoneal administration of hexythiazox at a dose of 3000 mg/kg bw reduced blood coagulation time in rats by approximately 30%; a dose of 1000 mg/kg bw had no significant effects. Hexythiazox at concentrations of 0.1, 1 or 10% w/v induced mild haemolysis of rabbit erythrocytes in vitro, which did not increase in severity with hexythiazoz concentration. A similar degree of haemolysis was observed with the positive control, 0.4% Tween 20 (Souma et al., 1985).

3. Observations in humans

Personnel in the plant manufacturing hexythiazoz receive a medical examination at least once per year. The examinations include a physical assessment and haematology, urine analysis, and clinical-chemistry investigations. No reports of adverse effects or any unusual patterns in the data were evident in summaries covering 1985 to 1987 (Motogi, 1987) and 1998 to 2007 (Kojima, 2008).

Hexythiazox has not been linked to adverse effects in any epidemiological reports. A single poisoning incident has been reported in the Philippines, but no details were available (Panganiban, 2001).

Comments

Biochemical aspects

The absorption, distribution and excretion of [^{14}C]hexythiazox was rapid in rats at 10 mg/kg bw, but much slower at 880 mg/kg bw. The extent of absorption at 10 mg/kg bw was approximately 30% on the basis of the level of urinary excretion, but significantly lower at 880 mg/kg bw. The maximum plasma concentrations of radiolabel were observed about 3–4 h after administration of the lower dose. The elimination half-life was about 10 h at 10 mg/kg bw, and was prolonged to about

20 h at 880 mg/kg bw, presumably reflecting saturation. This was confirmed by data on excretion. Most (about 60–90%, depending on the administered dose) of the radiolabel was excreted in the faeces within 3 days. About 10–20% of the administered dose was excreted as intact hexythiazox at the lower dose and 65–70% at the higher dose. The highest concentrations of tissue residues were found in fat, adrenals, liver and ovaries; the main component in fat was hexythiazox. Metabolism was extensive, but most of the radioactive material was not attributed to specific metabolites. The main metabolic reactions identified were hydroxylation of the cyclohexane ring and cleavage of the amide bond to the cyclohexane ring.

Toxicological data

Hexythiazox was of low acute toxicity when administered orally (LD_{50} > 5000 mg/kg bw), dermally (LD_{50} > 5000 mg/kg bw) or by inhalation (LC_{50} >2.0 mg/l air) routes. No deaths were reported in any of the submitted studies. Hexythiazox was not irritating to the skin of rabbits; was a slight, transient eye irritant and produced no evidence for skin sensitizing potential in a Magnussen and Kligman maximization study in guinea-pigs.

The toxicity of hexythiazox given as repeated doses has been investigated in mice, rats and dogs. Effects on body weight and the liver (which showed hypertrophy and, in some studies, necrosis) were seen relatively consistently. However, a number of other effects were seen at lower doses in dogs.

In a 28-day study in mice, body-weight gain was reduced, total cholesterol concentration in plasma was decreased, liver weights were increased and there were alterations in liver pathology at 1800 ppm and above. The NOAEL was 300 ppm, equal to 55 mg/kg bw per day.

In a 90-day study in rats, there were reductions in body-weight gain, alterations in erythrocyte parameters and increases in liver, kidney and adrenal weights and fatty degeneration of the adrenals at 500 ppm, equal to 36 mg/kg bw per day, and above. At 3500 ppm, the incidence of hepatocellular hypertrophy was increased in males and females and the incidence of glomerulonephrosis was increased in males. The NOAEL was 70 ppm, equal to 4.9 mg/kg bw per day.

In a preliminary 4-week study in groups of two male and two female dogs, the NOAEL was 125 ppm, equal to 5.5 mg/kg bw per day, on the basis of increased adrenal weights at 500 ppm and above. In a 1-year study in dogs, adrenal weights were increased and there was an increased incidence of adrenocortical hypertrophy at 500 ppm, equal to 13 mg/kg bw per day, and above. Also at 500 ppm, erythrocyte parameters and serum concentrations of inorganic phosphorus were reduced. At 5000 ppm, increased liver weights were associated with hepatocellular hypertrophy. The NOAEL in the 1-year study in dogs was 100 ppm, equal to 2.9 mg/kg bw per day.

The toxicity and carcinogenicity of hexythiazox has been investigated in long-term dietary studies in $B6C3F_1$ mice and F344 rats. In both studies, survival was > 70% in all groups at 2 years. Hexythiazox had no effect on survival in either study.

In mice, non-neoplastic findings included reduced body-weight gain, decreases in erythrocyte parameters, and increases in liver weight, hepatic necrosis, hepatic nodules and adrenal weights at 1500 ppm. At 250 ppm and above, there were reductions in leukocyte counts throughout the study and increases in the frequency of proteinaceous casts in the kidneys. Reductions in body-weight gain at 40 and 250 ppm were not considered to be biologically significant as the absolute body-weight values were similar to those of the historical controls. The incidences of hepatocellular adenoma and carcinoma were increased in males at 1500 ppm, but not statistically significantly ($p > 0.05$). In females the incidence of hepatocellular adenoma was increased significantly ($p = 0.033$) at 1500 ppm, but there was no change in the incidence of hepatocellular carcinoma in females. Low incidences of hepatoblastoma were seen in 3 out of 70 males at 1500 ppm, compared with a mean

incidence in historical controls of 0.2% (range, 0 out of 50 to 1 out of 50). Two of the three mice with hepatoblastoma also had hepatocellular adenoma and carcinoma, and the hepatoblastomas were considered to be part of the general pattern of liver tumours in these aged mice. The incidences of adenomas and carcinomas were related to age or duration of treatment as they were not increased in mice terminated or dying before week 78, the normal duration of a study of carcinogenicity in mice. The NOAEL for non-neoplastic effects was 40 ppm, equal to 6.7 mg/kg bw per day, and the NOAEL for carcinogenicity was 250 ppm, equal to 42 mg/kg bw per day.

In rats, non-neoplastic findings included increased adrenal weights and severity of vacuolation; withdrawn/swollen testes and the severity of chronic nephritis at 430 ppm and above, although the latter was statistically significant only in males at the highest dose at 12 months. At 3000 ppm, there were increases in liver weight and the incidence of hepatocellular cytoplasmic alterations, and increased ovary and spleen weights. The incidence of mammary-gland fibroadenoma was increased in males at 3000 ppm compared with values for historical controls, but not statistically significantly according to a pair-wise comparison with concurrent controls. The incidence of testicular interstitial-cell adenoma was increased at 3000 ppm at the interim 12-month kill relative to values for historical controls; the incidence in rats in the control group at study termination was > 90%, as is typical for the F344 strain. The size of interstitial-cell tumours and the occurrence of withdrawn/swollen testes might be related, but no specific measurements of tumour size were reported. The NOAEL for non-neoplastic effects was 60 ppm, equal to 3.2 mg/kg bw per day, and the NOAEL for carcinogenicity was 430 ppm, equal to 23 mg/kg bw per day.

Hexythiazox produced negative results in an adequate and extensive range of assays for genotoxicity in vitro and in vivo. An equivocal result in a non-standard study in yeast was not considered to be of significance when compared with the overall database.

The Meeting concluded that hexythiazox was unlikely to be genotoxic.

No investigations had been performed into potential mechanisms behind the increases in incidence of tumours.

On the basis of the negative results of tests for genotoxicity, the relatively high doses producing tumours and the NOAELs for non-neoplastic effects, the Meeting concluded that the increased incidences of tumours in rodents exposed to hexythiazox were likely to be threshold phenomena and that hexythiazox was unlikely to present a carcinogenic risk to humans at exposure levels associated with residues in food.

In a two-generation study of reproductive toxicity, parental rats showed toxicity consistent with other studies in rats; effects included: reduced body-weight gain, increased liver, kidney and adrenal weights. The NOAEL for adults was 400 ppm, equal to 24 mg/kg per day. The NOAEL for offspring was also 400 ppm, equal to 24 mg/kg bw per day, on the basis of reduced pup weights and associated changes in developmental landmarks at 2400 ppm. There were no effects on mating performance, gestation, litter size or pup survival at the highest dose tested, 2400 ppm, equal to 136 mg/kg bw per day.

In studies of developmental toxicity, hexythiazox did not induce specific malformations in either rats or rabbits. In the study of developmental toxicity in rats, the NOAEL for maternal toxicity (reduced body-weight gain) was 240 mg/kg bw per day; the NOAEL for developmental effects (reduced metatarsal ossification) was also 240 mg/kg bw per day. In the study of developmental toxicity in rabbits, there was no evidence of maternal toxicity at the highest dose tested, 1080 mg/kg bw per day, a dose that produced a slight increase in the number of fetuses with overlapping of the vertebral arches. The NOAEL for foetotoxicity in rabbits was 360 mg/kg bw per day.

The Meeting concluded that hexythiazox was not teratogenic and was not selectively toxic to the developing fetus.

No specific studies on neurotoxicity were submitted. Hexythiazox did not produce any neurotoxic effects in routine studies.

The effects of hexythiazox on the central nervous system, cardiovascular and respiratory system, skeletal muscle, isolated smooth muscle, intestinal motility, gastric secretion and on blood coagulation were studied in a general pharmacology study in a range of species. In these assays, hexythiazox did not demonstrate any pharmacological activity that would be of concern at dietary exposure levels.

A number of rat metabolites were investigated, all of which gave negative results in Ames tests. PT-1-2 (5-(4-chlorophenyl)-4-methyl-2-oxo-3-thiazolidine-carboxamide) and PT-1-3 (5-(4-chlorophenyl)-4-methyl-2-oxo-3-thiazolidine) were of moderate acute oral toxicity (LD_{50}s of about 1500 and 420 mg/kg bw, respectively). Other metabolites were of low acute oral toxicity (LD_{50}s of > 5000 mg/kg bw) in rats.

No reports of adverse effects or any unusual patterns in the data were evident in medical assessments of personnel from manufacturing plants spanning 20 years. Hexythiazox had not been linked to any epidemiological reports of adverse effects. A single poisoning incident was been reported in the Philippines, but no details were available.

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

Toxicological evaluation

An ADI of 0–0.03 mg/kg bw was established for hexythiazox based on the NOAEL of 3.2 mg/kg bw per day, identified in the 2-year study in rats on the basis of increases in fatty vacuolation of the adrenals in males and females, the severity of chronic nephritis and the incidence of swollen/withdrawn testes in males at 23 mg/kg bw per day and with a safety factor of 100. This was supported by the NOAEL of 2.9 mg/kg bw per day in the 1-year study in dogs.

The Meeting concluded that the establishment of an ARfD for hexythiazox was unnecessary on the basis of its low acute toxicity, the absence of developmental toxicity in rats and rabbits, the lack of evidence for any acute neurobehavioral effects, and the absence of any other toxicologically relevant effect that would be elicited by a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	40 ppm, equal to 6.7 mg/kg bw per day	250 ppm, equal to 42 mg/kg bw per day
		Carcinogenicity	250 ppm, equal to 42 mg/kg bw per day	1500 ppm, equal to 267 mg/kg bw per day
Rat	Two-year studies of toxicity and carcinogenicity [a]	Toxicity	60 ppm, equal to 3.2 mg/kg bw per day	430 ppm, equal to 23 mg/kg bw per day
		Carcinogenicity	430 ppm, equal to 23 mg/kg bw per day	3000 ppm, equal to 163 mg/kg bw per day
	Multigeneration study of reproductive toxicity[a]	Reproductive toxicity	2400 ppm, equal to 136 mg/kg bw per day[c]	—
		Parental toxicity	400 ppm, equal to 24 mg/kg bw per day	2400 ppm, equal to 136 mg/kg bw per day
		Offspring toxicity	400 ppm, equal to 24 mg/kg bw per day	2400 ppm, equal to 136 mg/kg bw per day

	Developmental toxicity[b]	Maternal toxicity	240 mg/kg bw per day	2160 mg/kg bw per day
		Embryo and fetal toxicity	240 mg/kg bw per day	2160 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	1080 mg/kg bw per day[c]	—
		Embryo and fetal toxicity	360 mg/kg bw per day	1080 mg/kg bw per day
Dog	One-year study of toxicity[a]	Increased adrenal weight and adrenal hypertrophy	100 ppm, equal to 2.9 mg/kg bw per day	500 ppm, equal to 13 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.03 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 hexythiazox

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid: $Cm_{ax,}$ 3–4 h; limited, about 30%, based on concentrations in urine.
Distribution	Extensive; highest concentrations in fat, liver, adrenals and ovaries.
Potential for accumulation	Slight, some persistence of low concentrations of hexythiazox in fat
Rate and extent of excretion	Rapid, > 70% in 24 h and relatively extensive, > 90% in 3 days
Metabolism in animals	Extensive but not fully identified. Major reactions are hydroxylation and cleavage of the amide bond to the cyclohexane ring
Toxicologically significant compounds (animals, plants and environment)	Hexythiazox and metabolites PT-1-2 and PT-1-3

Acute toxicity

Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 2 mg/l air (highest technically achievable), 4 h, whole body
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slight transient irritation
Guinea-pig, skin sensitization	Not a sensitizer (Magnussen & Kligman)

Short-term studies of toxicity

Target/critical effect	Reduced body-weight gain; increased liver and adrenal weight and associated pathology changes.
Lowest relevant oral NOAEL	2.9 mg/kg bw per day (1-year study in dogs; 100 ppm)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEL	No data

Genotoxicity

	No genotoxic potential in vitro or in vivo

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Body-weight gain, hepatotoxicity, adrenal fatty vacuolation, nephritis; testes (rat); haematology (mice).
Lowest relevant NOAEL	3.2 mg/kg bw per day (2-year study in rats, 60 ppm)
Carcinogenicity	None relevant at levels of human dietary exposure

Reproductive toxicity

Reproduction target/critical effect	No adverse effect on reproduction
Lowest relevant reproductive NOAEL	136 mg/kg bw per day (rats, 2400 ppm, highest dose tested)
Developmental target/critical effect	Reduced ossification of metatarsals (rats) Increase in overlapping of the vertebral arches (rabbits)
Lowest relevant developmental NOAEL	240 mg/kg bw per day (rats)

Neurotoxicity/delayed neurotoxicity

Acute neurotoxicity	No specific studies; no indications from routine studies
Other toxicological studies	
	Screen for pharmacological activity did not identify any potent activity. All rat metabolites tested were negative in Ames tests. Two, PT-1-2 & PT-1-3, were of moderate acute oral toxicity; other metabolites were of low acute oral toxicity in rats

Medical data

	No adverse reports from health surveillance of production plant workers. No reports of adverse findings in the published literature. One report of a poisoning case in the Philippines.

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.03 mg/kg bw	Rat, 2-year study	100
ARfD	Unnecessary	—	—

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MANDIPROPAMID

First draft prepared by
C. Adcock[1] & L. Davies[2]

[1]Health Evaluation Directorate,
Pest Management Regulatory Agency, Health Canada, Canada; and
[2]Chemical Review, Australian Pesticides and Veterinary Medicines Authority,
Canberra, Australia

Explanation

Mandipropamid is the ISO approved name for 4-chloro-*N*-[2-[3-methoxy-4-(2-propynyloxy) phenyl]ethyl]-α-(2-propynyloxy)-benzeneacetamide (CAS No. 374726-62-2). It is a new fungicide that belongs to the subset mandelamides in the class carboxylic acid amides. The proposed fungicidal

mode of action is by inhibition of phospholipid biosynthesis. Mandipropamid has not been evaluated previously by the JMPR and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR). All the pivotal studies contained certificates of compliance with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

The toxicokinetics of technical-grade mandipropamid have been studied in rats given oral doses of radiolabelled test material. Summaries of the relevant data are presented below.

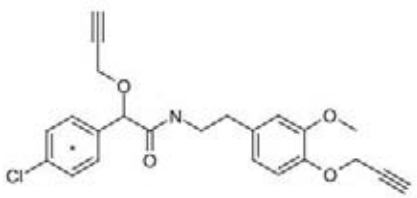
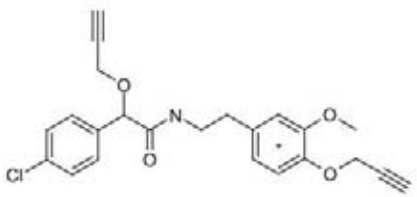
1.1 Absorption, distribution and excretion

Rats

In studies of metabolism, groups of three or four male and three to four female Alpk:APfSD (Wistar-derived) rats were given [^{14}C]mandipropamid (radiochemical purity, 95.2–96.8%) as a single oral dose at 3 or 300 mg/kg bw or as multiple doses of 3 mg/kg bw per day by gavage in 1% w/v aqueous carboxymethylcellulose for up to 14 days. Results were evaluated after treatment with [methoxyphenyl-U-^{14}C]mandipropamid or [chlorophenyl-U-^{14}C]mandipropamid (see Table 1). Bile-duct cannulation and tissue time-course studies were also performed.

Of the administered dose, 67–74% was absorbed by the rats at 3 mg/kg bw and 30–45% in the rats at 300 mg/kg bw, based on the sum of the radioactive residues found in the urine, bile, carcass and cage-wash 48 h after dosing. There was no clear difference in compound absorption between males and females. Absorption was decreased at the higher dose, suggesting saturation of the absorption kinetics. The T_{max} in blood at the lower dose was 8.5 h in males and 4.5 h in females, and at the higher dose was 24 h in males and 10 h in females. These data suggested that the rate of absorption is greater in females than in males, while the extent of the absorption is similar in males and females, and that the extent and rate of absorption was greater at the lower dose than at the higher dose.

Table 1. Radiolabelled forms of mandipropamid used in studies of absorption, distribution, metabolism and excretion: chemical structure and position of the radiolabel

Radiolabelled form	Structure and position of label
[Chlorophenyl-U-^{14}C]mandipropamid	
[Methoxyphenyl-U-^{14}C]mandipropamid	

* Position of radiolabel

Total recoveries at 168 h after dosing were 88–99% of the administered dose. The administered dose was mostly eliminated within 48 h. Apart from females at 3 mg/kg bw, most of the administered dose was excreted in the faeces regardless of the position of the radiolabel. In the females at 3 mg/kg bw, the amount excreted in the faeces was similar to that excreted in the urine. Regardless of sex, radiolabel position or dose, the amount of radiolabel isolated from the exhaled air was 0.16% or less of the administered dose, and the amount of radiolabel remaining in the body after 168 h was < 1.1% of the administered dose. In the multiple-dose study (methoxyphenyl label only), the excretion profile on day 2 after a single dose was similar to that on day 15 after 14 doses, indicating that pre-treatment did not influence the excretion profile. Elimination after 48 h in the bile was high (55–73% of the administered dose) at 3 mg/kg bw, but was only 22–28% of the administered dose at 300 mg/kg bw (at this dose, there were corresponding increases in the residues found in the faeces of males and urine of females).

The highest concentration of radiolabel was found in the liver at the initial measurement and all following measurements. At 8 h after dosing, concentrations were 0.64–1.25 µg equivalents/g (0.89 µg equivalents/g on day 4 in the multiple-dose study) at 3 mg/kg bw and 27–46 µg equivalents/g at 300 mg/kg bw. Concentrations of radiolabel in the liver of males were approximately double those in females. The second highest concentrations were usually found in the kidneys. More radiolabel was isolated in the plasma than in whole blood. The profile of tissue distribution was thus generally similar regardless of dose, radiolabel position, or sex; however, there were quantitative differences. Tissue concentrations did not increase proportionally with dose. An increase in dose of 100-fold resulted in a increase in concentrations in the liver of 17–56-fold. Results from the multiple-dose study indicated that bioaccumulation did not occur in any sampled tissue. Half-life values for the elimination of radiolabel from the blood were 18.4–20.2 h at 3 mg/kg bw and 24.8–32.7 h at 300 mg/kg bw (Roberts, 2005a, 2005b; Silcock & Duerden, 2005; Wake, 2005).

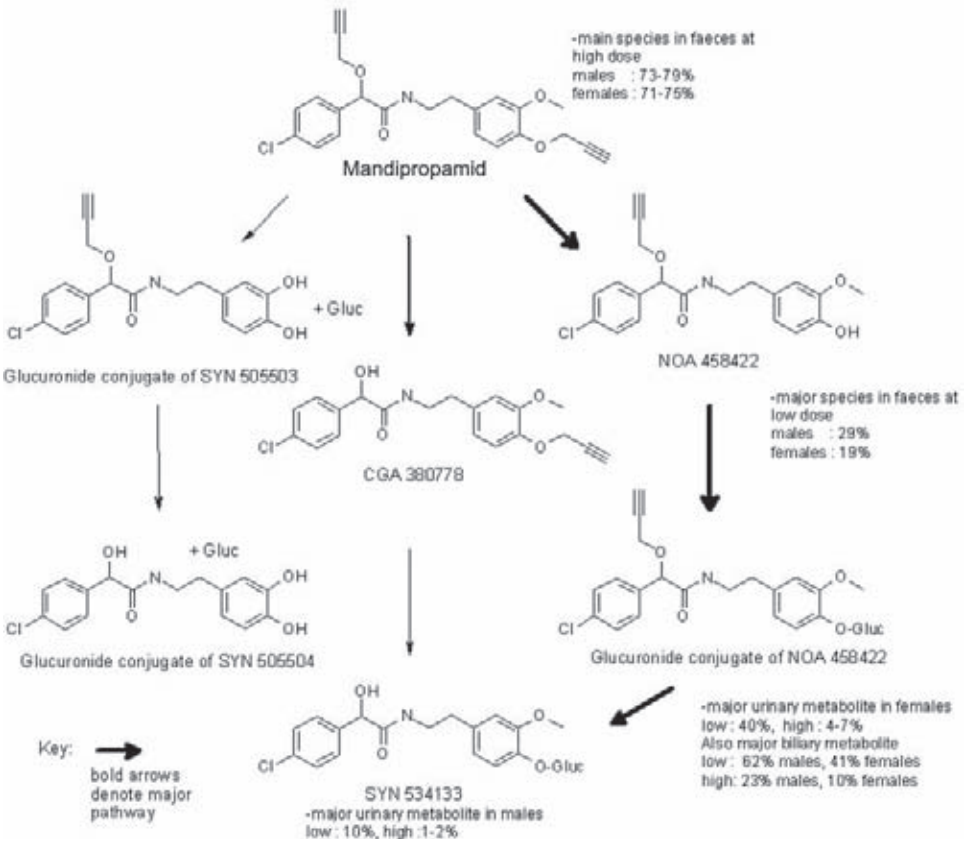
1.2 Biotransformation

Rats

In the main study of biotransformation in rats, identified compounds accounted for 65.7–93.5% of the administered dose in each group; however, 6–14% of the administered dose was unaccounted for after 168 h. Differences in the metabolite profile were found on the basis of sex, dose, and radiolabel position as described below and shown in Figure 1.

Parent compound and the following metabolites were present in the urine, faeces or bile at concentrations of 5% or greater of the administered dose in rats at 3 or 300 mg/kg bw: NOA 458422, NOA 458422 glucuronide, SYN 534133, and CGA 380778. The quantity of each metabolite isolated in males and females at 3 mg/kg bw after sampling for 168 h differed. The amount of NOA 458422 glucuronide was almost three times greater in females than in males, while lesser amounts of parent and NOA 458422 were isolated in females than in males. Increasing the dose resulted in increasing amounts of radiolabel isolated as parent. In the rats treated with [chlorophenyl-U-^{14}C] mandipropamid, only the parent was isolated in the faeces at > 5% of the administered dose, but other compounds were also isolated in rats treated with [methoxyphenyl-U-^{14}C]mandipropamid. Thus, there were differences in the metabolite profile attributable to sex and dose. Each isolated unidentified compound accounted for < 5% of the administered dose. The major metabolic transformations involved loss of one or both of the propargyl groups followed by glucuronidation and *O*-demethylation (Wake, 2005).

Figure 1. Proposed metabolic pathway of mandipropamid in rats



Dermal absorption

In a study of dermal penetration, [Ethyl-1-^{14}C]mandipropamid (radiochemical purity, > 97.9%) was applied to the skin (10 cm^2) of groups of four male Alpk:AP$_f$SD rats. Nominal doses were 0.00152, 0.0076, or 2.54 mg/cm^2 skin, with water and/or a commercial formulation containing 23.7% (w/w) mandipropamid as a 250 g/l suspension concentrate (A12946B). The highest dose mimicked a formulation concentrate, and was included to assess exposure to mixer/loaders. The lower doses were aqueous dilutions representing typical in-use spray strength dilutions of 1/333 and 1/1667 v/v. The exposure duration was 6 h, after which four males per dose were sacrificed. The remaining groups of four males were sacrificed at 24, 72, or 120 h after dose application.

Recovery of the applied dose (mass balance) was 96–112%. Minimal absorption, based on the sum of residues in urine, faeces, cage-wash, gastrointestinal tract with contents, residual carcass, and blood, was observed (< 0.17 to 3.44% of the applied dose). Most of the administered dose (91–105%) was recovered from the skin wash at 6 h; 0.03–0.47% of the applied dose was retained at the application site, and 0.02–0.77% was found in the *stratum corneum*.

The greatest amount of absorption was noted in the 1/1667 aqueous dilution 114 h after the 6 h exposure interval. At this time 3.44% of the applied dose was absorbed. The amount available for absorption in the skin at the application site was less than the limit of detection (LOD) in all four rats (< 0.11%) with an additional 0.53% isolated in the stratum corneum (Silcock, 2005).

In a non-guideline study of dermal penetration in vitro, [ethyl-1-14C]mandipropamid (radiochemical purity, > 95%) was applied to excised rat skin in vitro. The formulation suspension

concentrate was included to assess exposure to mixer/loaders. The aqueous dilutions represented a typical in-use spray strength dilution of 1/333 and 1/1667 v/v. The applied nominal doses were 1.35, 6.69, or 2538 µg/cm2 skin. Skin was used from freshly killed male Wistar rats. Skin membrane discs of approximately 3.3 cm in diameter were prepared from at least two rats, and were mounted in diffusion cells with an exposed membrane area of 2.54 cm2. Each of the three doses were applied to the skin in a dose volume of 10 µl/cm2 (n = 4–6) for a duration of 6 or 24 h. The cells were not occluded for the duration of exposure. Samples of receptor fluid were taken before treatment and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 h after dose application for those cells assigned an exposure of 24 h, or at 6 h for those cells assigned an exposure of 6 h. The original volume of the receptor fluid was restored by adding fresh receptor fluid after sampling. The test substance absorbed was considered to be the mandipropamid detected in the receptor fluid.

Recovery of the applied radioactivity was 99–105%. Absorption was poor. In the 1/1667 v/v spray dilution after 24 h of exposure (recovery, 104%), 13.1% of the radioactive compound was absorbed and 89.1% was found in the skin wash. At the other exposure conditions, < 0.03% to 6.00% of the radioactive compound was absorbed and 93.5–100% was isolated in the skin wash. The absorption rate was greatest during the first hour of exposure (0.05–0.06 µg/cm2 per h for the aqueous spray dilutions). Absorption rates over the 24 h of exposure were < 0.04 µg/cm2 per h in the concentrate formulation (concentrations measured below the quantifiable limit) and only 0.01 µg/cm2 per h in the aqueous spray dilutions. The amount absorbed as a percentage of applied dose decreased with increasing dose (Davies, 2005a).

In a non-guideline study of dermal penetration in vitro, [ethyl-1-14C]mandipropamid (radiochemical purity, > 95%) was applied to excised human skin in vitro. The formulation concentrate was included to assess exposure to mixer/loaders. The aqueous dilutions represented a typical in-use spray strength dilution of 1/333 and 1/1667 v/v. The applied nominal doses were 1.35, 6.69, or 2538 µg/cm2 skin. Skin membrane discs of approximately 3.3 cm in diameter were prepared from at least two humans, and were mounted in diffusion cells with an exposed membrane area of 2.54 cm2. Each of the three doses were applied to the skin in a dose volume of 10 µl/cm2 (n = 5–6) for a duration of 6 or 24 h. Samples of receptor fluid were taken before treatment and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 h after dose application for those cells assigned an exposure of 24 h or at 6 h for those cells assigned an exposure of 6 h. The test substance absorbed was considered to be the mandipropamid detected in the receptor fluid.

Recovery of the applied radioactivity was 95–102%. Absorption was minimal. Absorption was < 0.04% to 0.77%, and 93.9–102% of the applied radiolabel was isolated in the skin wash. The absorption rate was greatest during the first 1–2 h of exposure (0.003–0.004 µg/cm2 per h in the aqueous spray dilutions). Absorption rates over the 24 h of exposure were < 0.04 µg/cm2 per h in the concentrate formulation (concentrations measured below the quantifiable limit) and ≤ 0.001 µg/cm2 per h in the aqueous spray dilutions. The amount absorbed as a percentage of applied dose decreased with increasing dose (Davies, 2005b).

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with mandipropamid technical are summarized in Table 2. All studies complied with GLP.

(a) Lethal doses

In a study of acute oral toxicity, three female Sprague-Dawley rats were given a single oral dose of mandipropamid (purity, 96.5%) at 5000 mg/kg bw using the up-and-down procedure. The rats were inspected for mortality and clinical abnormalities during the first hours after dosing and at least once per day for up to 14 days thereafter. Body weights were obtained before dosing and again on days 7 and 14. A necropsy examination was performed on all rats.

All rats survived and gained weight throughout the study. One rat exhibited anogenital staining at 5 h after dosing. No other clinical abnormalities were observed. No gross abnormalities were observed at necropsy. The oral median lethal dose (LD_{50}) for females was estimated to be > 5000 mg/kg bw (Moore, 2004).

In a study of acute dermal toxicity, five male and five female Sprague-Dawley rats were exposed dermally to mandipropamid (purity, 96.5%) at a dose of 5050 mg/kg bw. On the day before study initiation, the dorsal trunk area was clipped free of fur. On the day of study initiation, each individual dose of the test substance was moistened with "a sufficient amount of corn oil (0.75 ml/g test substance)" and applied to the test site in a thin, uniform layer, and covered with a 2 × 4 inch gauze patch. The patch was secured with non-irritating adhesive tape. The trunk of each rat was wrapped with vet wrap and secured with non-irritating adhesive tape to prevent ingestion of the test material. After 24 h, all binding materials were removed. The test sites were washed with tap water at room temperature and a clean cloth was used to remove any residual test substance. The rats were observed for signs of toxicity or clinical abnormalities at least three times on the day of dosing (day 0) and at least once per day thereafter for 14 days. Body weights were measured just before dosing and again on days 7 and 14. Observations for dermal irritation at the test site were taken on days 1, 4, 7, 11 and 14. A necropsy examination was performed on all rats tested.

All rats survived to study termination. No clinical abnormalities or skin irritation were observed. Body weight was unaffected by mandipropamid. No gross abnormalities were observed at necropsy. There were no deaths at 5050 mg/kg bw (Kuhn, 2005).

In a study of acute toxicity on inhalation, five male and five female Alpk:AP_fSD rats were exposed "nose-only" via inhalation to mandipropamid (purity, 96.5%) at a gravimetric concentration of 5.19 ± 0.55 mg/l air. The rats were observed for clinical signs frequently during and at the end of exposure and daily until study termination. Body weights were recorded on study days –1, 1, 8 and 15. A necropsy examination was performed on all rats tested.

All rats (five out of five males and five out of five females) survived to study termination. Clinical abnormalities observed during exposure included wet fur, stains around the nose, slight salivation, and respiratory-tract irritation (increased breathing depth). Immediately after exposure, the rats continued to exhibit wet fur and stains around the nose. Some rats also exhibited chromodacryorrhoea, slight salivation, and irritation of the upper respiratory tract (abnormal respiratory noise). All females recovered by study day 2 and males by study day 4. All rats gained normal body weight by

Table 2. Acute toxicity with mandipropamid[a]

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Reference
Rat	Sprague-Dawley	Females	Oral	> 5000	—	Moore (2004)
	Sprague-Dawley	Males & females	Dermal	> 5050	—	Kuhn (2005)
	Alpk:AP_fSD	Males & females	Inhalation	—	> 5.19	Kilgour (2003)

[a] Technical mandipropamid of purity 96.5% was used in all the studies of acute toxicity.

study termination. At necropsy, one male exhibited a pelvic dilation of the kidney. The study author stated that this is common for the age and strain of the rat used and did not attribute this observation to administration of the test substance. No other abnormalities were observed at necropsy. The median lethal concentration (LC_{50}) was > 5.19 ± 0.55 mg/l air (Kilgour, 2003).

(b) Ocular irritation

Rabbits

In a study of primary eye irritation in New Zealand White rabbits, two males and one female received approximately 100 mg of undiluted mandipropamid (purity, 98.5%) into the conjunctival sac of the left eye. The right eyes were left untreated to serve as negative controls. Ocular irritation was graded according to the Draize scale at 1 h and additionally at 1, 2, 3, 4 and 7 days after instillation.

No corneal opacity was observed during the study. Iritis was observed in two out of three rabbits at 1 h only. Positive signs of conjunctivitis were observed in three out of three rabbits at 1 h, resolving within 4 days. Mandipropamid was minimally irritating to the eyes of New Zealand White rabbits (Johnson, 2004a).

(c) Dermal irritation

Rabbits

In a study of primary skin irritation in New Zealand White rabbits, two males and one female received approximately 500 mg of mandipropamid (purity, 98.5%) applied topically to shaved skin sites on the back. The test site was covered with gauze and a sheet of rubber for 4 h after which all coverings were removed and the site was washed with water and wiped dry with clean tissue paper. Each test site was evaluated for irritation potential according to the Draize scale at 1 h and additionally at 1, 2, 3, 4 and 7 days after application. Very slight (grade 1) erythema was observed in one out of three rabbits at 1 h, resolving within 7 days. This rabbit also exhibited slight desquamation. No oedema was observed during the study. Mandipropamid was minimally irritating to the skin of New Zealand White rabbits (Johnson, 2004b).

(d) Dermal sensitization

In a study of dermal sensitization with mandipropamid (purity, 96.5%), groups of four male CBA/Ca/Ola/Hsd mice were tested for a dermal sensitization response using the local lymph node assay. The mice received mandipropamid as 10%, 25%, or 50% w/v preparations in dimethylformamide (DMF). A study using the positive control hexylcinnamaldehyde was conducted within 6 months of the main study. Approximately 25 µl of a 1%, 3% or 10% w/v preparation of hexylcinnamaldehyde in acetone was applied, and a vehicle-control group was similarly treated using acetone. The test substance produced a stimulation index of < 3 in all groups of mice. Therefore, mandipropamid was not considered to be a sensitizer (defined as producing a positive response) (Johnston, 2004c).

Table 3. Irritation and skin sensitization potential of mandipropamid

Species	Strain	Sex	End-point	Purity (%)	Result	Reference
Rabbit	NZW	M/F	Eye irritation	98.5	Minimally irritating	Johnson (2004a)
Rabbit	NZW	M/F	Skin irritation	98.5	Minimally irritating	Johnson (2004b)
Mouse	CBA/CA/Ola/Hsd	M	Skin sensitization (local lymph node assay)	96.5	Not sensitizing	Johnston (2004c)

F, females; M, males; NZW, New Zealand White.

2.2 Short-term studies of toxicity

(a) Oral administration

Rats

Groups of 10 male and 10 female Alpk:APfSD rats were given diets containing mandipropamid (purity, 96.5%) at a concentration of 0, 100, 500, 3000 or 5000 ppm (equal to 0, 8, 41, 260 or 435 mg/kg bw per day in males and 0, 9, 45, 260 or 444 mg/kg bw per day in females) for 90 days.

No adverse treatment-related effects were observed on mortality, clinical signs, neurological evaluation, food consumption, ophthalmoscopic examinations, haematology, urine analysis, or gross pathology. Decreased body weights were observed in the males at 3000 ppm during weeks 2–14 (decrease, 1–10%) and at 5000 ppm during weeks 2–5, 11, and 13–14 (decrease, 2–7%). Differences in cumulative body-weight gains generally paralleled the differences in body weights. Decreases of 11–28% in body-weight gain were noted in the males at dietary concentrations of 3000 ppm or greater during weeks 1–7, 7–14, and overall (weeks 1–14). Decreased food utilization was noted in males at 3000 ppm or greater during weeks 1–4, 5–8, 9–13, and 1–13 (decrease, 9–29%), except in males 5000 ppm males during weeks 5–8. Indications of slight hepatotoxicity were observed.

Plasma gamma-glutamyl transferase was increased in females at dietary concentrations of 3000 ppm or greater (increase, 58–105%). Absolute and relative liver weights were increased by 14–36% in males and females at 3000 ppm or greater. Minimal to slight periportal hypertrophy/eosinophilia in the liver was noted in males at 5000 ppm (8 out of 10) and the females at 3000 ppm or greater (10 out of 10 treated) compared with 0 out of 10 in the controls and other dose groups. In the erythropoetic system, toxicity was demonstrated as decreases in a number of erythrocyte parameters (see Table 4), and increased kidney weight (males) and an increased incidence of tubular basophilia at the highest dose only were indicative of renal effects.

The no-observed-adverse-effect level (NOAEL) was 500 ppm, equal to 41 mg/kg bw per day in males and 45 mg/kg per day in females, on the basis of decreased body weight, body-weight gain and food utilization at 3000 ppm (Pinto, 2005a).

Table 4. Selected haematology findings in rats given diets containing mandipropamid for 90 days (means)

Parameter (mean)	Dietary concentration (ppm)									
	Males					Females				
	0	100	500	3000	5000	0	100	500	3000	5000
Haemoglobin (g/dl)	14.8	15.1	14.7	14.7	14.2*	15.3	15.1	15.0	14.5**	14.2**
Haematocrit	0.465	0.474	0.464	0.469	0.456	0.473	0.468	0.463	0.454*	0.445**
Mean cell volume (fl)	51.4	51.4	51.0	49.9**	48.9**	53.9	54.0	52.9*	52.0**	51.7**
Mean cell haemoglobin (pg)	16.3	16.4	16.2	15.6**	15.3**	17.4	17.4	17.1	16.6**	16.5**
Mean cell haemoglobin concentration (g/dl)	31.7	31.8	31.7	31.3*	31.2*	32.3	32.3	32.4	31.8*	31.9*

From Pinto (2005a)
* $p < 0.05$ (Student t-test, two-sided)
** $p < 0.01$ (Student t-test, two-sided)

Dogs

In a 90-day study of oral toxicity, groups of four male and four female beagle dogs were given capsules containing mandipropamid (purity, 96.5%) at a concentration of 0, 5, 25, 100, or 400 mg/kg bw per day.

There were no mortalities and no adverse effects of treatment on clinical observations, food consumption, ophthalmoscopy, haematology, urine analysis, or gross pathology. There were no treatment-related effects on any parameter at 5 or 25 mg/kg per day. Body-weight gains and body weights were decreased in females at 100 mg/kg per day beginning at week 8 and in dogs at 400 mg/kg per day beginning at week 3; however, these decreases were not considered to be adverse because they were minimal (body weights were only decreased by 2–5% and body-weight gains by 9–15%). Note that similar decreases (≤ 12%) in body weights were observed throughout the long-term study of toxicity (Brammer, 2005b) at 400 mg/kg per day, but were associated with more substantial decreases of 23–56% in body-weight gains for weeks 1–13 than in this short-term study.

The liver was a target organ for toxicity. At 400 mg/kg per day, increases were reported in cholesterol concentration (increase, 27–49%), alkaline phosphatase activity (increase, 37–113%), and alanine aminotransferase (ALT) activity (increase, 43–238%, not significant at week 4) were observed in males and females throughout the study, compared with controls. At 100 mg/kg per day, cholesterol concentration and alkaline phosphatase activity were only increased by 22–53% and were not consistently observed throughout the study. ALT, the most definitive indicator of liver toxicity, was only increased by 35–37% at week 13.

Increases (10–19%) in absolute, and relative (to body weight) liver weights were observed in males and females at doses of 100 mg/kg bw per day or greater. At 100 mg/kg bw per day, minimal pigmentation of the hepatocytes and of the Kupffer cells were observed in one out of four males and two out of four females, compared with zero controls. At 400 mg/kg bw per day, the incidence of this finding increased in all dogs (four out of four males and four out of four females), and the severity increased from minimal at 100 mg/kg bw per day to minimal to slight at 400 mg/kg bw per day. It was stated that this brown pigment had a predominantly centrilobular distribution and was negative for haemosiderin and lipofuscin. It was bi-refringent when viewed under polarized light and appeared to be consistent with porphyrin. Minimal centrilobular hepatocyte vacuolation was noted in females at 100 mg/kg bw per day (one out of four) and females at 400 mg/kg bw per day (three out of four) compared with 0 out of 4 controls. The microscopic findings at this dose were minimally increased in severity and/or incidence over controls.

The NOAEL was 25 mg/kg bw per day on the basis of evidence of liver toxicity (increased cholesterol, alkaline phosphatase activity, ALT activity, liver weights and microscopic pigment in the hepatocytes and Kupffer cells in males and females and centriolobular hepatocyte vacuolation in females) (Brammer, 2005a).

In a 1-year study, groups of four male and four female beagle dogs were given capsules containing mandipropamid (purity, 96.54%) at a dose of 0, 5, 40 or 400 mg/kg bw per day.

There were no mortalities and no effects of treatment on ophthalmoscopy, haematology, urine analysis, or gross pathology. There were no treatment-related effects on any parameter in dogs at 5 mg/kg per day. One female at 40 mg/kg bw per day was slightly thin beginning at week 6, and one female at 400 mg/kg per day was slightly to moderately thin beginning at week –2. Additionally at 400 mg/kg bw per day, two females exhibited slight to moderate salivation during dosing with the capsule (83 incidences beginning at week 3) and at non-dosing observation intervals (96 occasions beginning at week 1). These clinical findings continued until study termination. At 400 mg/kg bw per day, decreases of 1–12% in body weights were observed in males and females throughout the study. Body-weight gains for weeks 1–13 were decreased by 23% compared with controls in the males and by 30% in the females. Body-weight gains for the study overall (weeks 1–53) remained decreased at

this dose in males (decrease, 15%) and females (decrease, 45%) (Table 5). There were no effects on body weight at 5 or 40 mg/kg bw per day. Additionally, in females at 400 mg/kg bw per day, minor (≤7% compared with controls) but consistent (43 out of 52 weeks) decreases in food consumption were observed.

The liver was the target organ for toxicity. At doses of 40 mg/kg bw per day or greater, alkaline phosphatase activity was increased throughout the study in males (57–174%) and females (27–273%). These increases attained significance, except in females at 40 mg/kg bw per day at week 13. In the males, ALT activity was increased throughout the study at 40 mg/kg bw per day (89–150%) and 400 mg/kg bw per day (315–445%). ALT activity was also increased (182–352%) in females at 400 mg/kg per day throughout the study, attaining significance at week 52. Increases in absolute liver weight (7–15%), relative (to body weight) liver weight (20–27%), and liver weight adjusted for terminal body weight[1] (16–19%) were observed in males and females at 400 mg/kg bw per day, with adjusted liver weight in the males attaining significance. At 40 mg/kg bw per day, minimal increased pigment in the liver was observed in two out of four males and one out of four females, compared with zero out of four controls. At 400 mg/kg bw per day, the incidence of this finding increased to three out of four males and three out of four females, and the severity was minimal to moderate in the males and minimal to slight in the females. It was stated that this pigment appeared to be consistent with porphyrin.

The NOAEL was 5 mg/kg per day on the basis of liver toxicity (increased incidence and severity of pigment, increased activities of alkaline phosphatase and ALT) at 40 mg/kg bw per day (Brammer, 2005b).

Table 5. Comparison of body weights of dogs given capsules containing mandipropamid for up to 1 year

Week[a]	Body weight (kg)							
	Dose (mg/kg bw per day)							
	Males				Females			
	0	5	40	400	0	5	40	400
1	9.20	9.30	8.88	9.18	7.95	8.03	7.80	7.73
4	9.94	9.85	9.71	9.57*	8.34	8.30	8.24	8.23
8	10.65	10.64	10.35	10.09*	8.79	8.67	8.64	8.43
12	11.15	11.23	10.77	10.60*	9.06	9.12	8.99	8.58
18	11.76	11.76	11.26	11.17	9.61	9.58	9.46	8.87*
24	12.06	12.13	11.31	11.47	9.82	9.91	9.66	9.02
48	12.38	12.85	11.51	11.91	10.38	10.54	10.17	9.28
53	12.60	13.02	11.68	12.06	10.51	10.78	10.39	9.22

From Brammer (2005b)

[a] Adjusted mean values shown for week 4 onwards.

* $p < 0.05$ (Student t-test, two-sided)

** $p < 0.01$ (Student t-test, two-sided)

1 Group mean organ weight corrected for intergroup differences in the group mean terminal body weight. The adjusted means are calculated statistically using analysis of covariance. The size of the adjustment is determined by the size of the intergroup differences in the group mean terminal body weight and the strength of the relationship between organ weight and terminal body weight.

(b) Dermal administration

Rats

In a study of dermal toxicity, groups of 10 male and 10 female Wistar rats received mandipropamid (purity, 96.5%) at a dose of 0, 250, 500, or 1000 mg/kg bw per day (limit dose) applied to the shaved skin for 6 h per day for 5–6 days per week (a total of 21 total doses) during a 28-day period.

No compound-related effects were observed on mortality, clinical signs of toxicity, body weight, body-weight gain, food consumption, a functional observational battery (FOB), motor activity, ophthalmoscopic examinations, haematology, clinical chemistry, urine analysis, absolute or relative organ weights, or gross or microscopic pathology in males or females. There was an increased incidence of slight dermal irritation (erythema, oedema, and desquamation of the application site) at doses of 250 mg/kg bw per day and above. The erythema and oedema were generally observed throughout the study and the desquamation was observed between days 3–15 in the treated rats.

The NOAEL was 1000 mg/kg per day, the highest dose tested (Lees, 2005).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female C57BL/10J$_f$CD-1 mice were given diets containing mandipropamid (purity, 96.5%) at a concentration of 0, 100, 500, or 2000 ppm (equal to 0, 10.6, 55.2 or 222.7 mg/kg bw per day in males and 0, 13.2, 67.8 or 284.6 mg/kg bw per day in females) for up to 80 weeks.

No adverse treatment-related effects were observed on mortality, food consumption, haematology (i.e. leukocyte differential), gross pathology, or histopathology. An increased frequency of circling behaviour was observed in females at 500 and 2000 ppm. However, this behaviour was only slightly increased in incidence at 2000 ppm compared with controls (two, one, two, and four mice at 0, 100, 500 and 2000 ppm, respectively.

Dose-related increases in the number of mice with a torn left ear were observed in males and females at 500 and 2000 ppm. The number of mice with a torn right ear was minimal in incidence (≤ 1 mouse per group) and did not show a pattern with dose. It was stated that the mice were identified by ear tags, and it is likely that these identification tags were placed in the left ear. The mice were housed together in groups and could have removed the tags (and selectively torn the left ear) of their cage-mates via fighting or other interactions. However, this would not explain why the incidence of this finding was dose-related. The possibility that the test substance increased fighting or some other behaviour that led to the left ear being torn cannot be ruled out. However, because there were no dose-related incidences in scabs or other signs of fighting and because there were no clinical signs of neurotoxicity in this study or in other studies of acute neurotoxicity or short-term studies of neurotoxicity in rats, the Meeting considered that the toxicological importance of this finding was equivocal.

At 2000 ppm, body weights were slightly decreased in males generally from weeks 19–35 and in the females during weeks 4, 5, and 13–81. Cumulative weekly body-weight gains were decreased at this dose in males during weeks 19–81 and in females during weeks 4–6 and 11–81. The decreased body-weight gains attained statistical significance throughout the study, except at week 81 in males and at week 79 in females. Food utilization was decreased in males during weeks 9–13 (decrease, 20%) and weeks 1–13 (decrease, 5%) and in the females for weeks 1–4 (decrease, 12%) and reflected the decrease in body weights at this dose. Absolute, relative-to-body-weight, and adjusted-for-initial-body-weight liver weights were increased in males at 500 ppm and in males and females at 2000 ppm. These increases were statistically significant, except for absolute weights in males at 2000 ppm (relative liver weights were not examined statistically). In the absence of any microscopic findings in the liver, the relatively small increases in liver weights were not considered to be adverse,

but were likely to be an adaptive response to the presence of the test substance. At the doses tested, there were no effects of treatment on the incidence or time to onset of any tumour types. Therefore, there was no evidence of a carcinogenic effect. Dosing was considered to be adequate on the basis of decreased body weight and body-weight gain.

The NOAEL was 500 ppm, equivalent to 55 mg/kg bw per day in males and 68 mg/kg bw per day in females, on the basis of decreased body-weight gain in males and females and food utilization in males at 2000 ppm (Milburn, 2005a).

Rats

In a long-term study of combined toxicity and carcinogenicity, groups of 52 male and 52 female Alpk:AP_fSD rats were given diets containing mandipropamid (purity, 96.5%) at a concentration of 0, 50, 250, or 1000 ppm (equal to 0, 3.0, 15.2 or 61.3 mg/kg bw per day in males and 0, 3.5, 17.6 or 69.7 mg/kg bw per day in females) for up to 2 years. Additional groups of 12 males and 12 females were treated similarly and terminated after 1 year.

No treatment-related effects were observed on mortality, clinical signs, neurological evaluation, food consumption, ophthalmoscopic examination, haematology, clinical chemistry, urine analysis, or organ weights. No treatment-related pathological findings were noted at 12 months. In males at 1000 ppm, decreased body weights were generally observed during weeks 2–15 and 67–103 (decrease, 1–6%). A similar effect was observed on cumulative body-weight gain (decrease, 3–7%). Overall (weeks 1–105) body-weight gain was decreased by 6% (not statistically significant). Food utilization was decreased during weeks 1–4, 5–8, 9–13, and 1–13 by 5–7% ($p \leq 0.01$; except not statistically significant at weeks 9–13). An increased incidence of a roughened surface was observed (13 out of 64 treated vs 5 out of 64 controls) in the kidney of males at 1000 ppm. The severity of chronic progressive nephropathy was increased in males receiving mandipropamid, with an increased incidence of moderate to marked severity (38, 41, 47 and 53% in the control group and at 50, 250 and 1000 ppm, respectively). Associated increases in the incidences of minimal to marked renal osteodystrophia fibrosa (19% treated vs 8% controls) and minimal to marked parathyroid hyperplasia (28% treated vs 17% controls) were also noted. At the doses tested, there was no treatment-related increase in tumour incidence when compared with controls. Dosing was considered to be adequate on the basis of decreased body-weight gain and food utilization and increased nephrotoxicity in the males.

The NOAEL was 250 ppm, equivalent to 15.2 mg/kg bw.per day in males and 17.6 mg/kg bw per day in females, on the basis of decreased body-weight gain and food utilization and increased nephrotoxicity in males (Pinto, 2005b).

2.4 Genotoxicity

(a) In vitro

In three independent trials of an assay for reverse gene mutation, *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strains WP2P and WP2P*uvr*A were exposed to mandipropamid (purity, 96.5%) at concentrations of 0, 100, 200, 500, 1000, 2500, or 5000 µg/plate in dimethyl sulfoxide (DMSO) with or without metabolic activation from the S9 fraction derived from livers of male Sprague-Dawley rats induced with phenobarbital/β-naphthoflavone. The study was complied with OECD guideline 471. The standard plate incorporation method was used in all except one trial (in the presence of metabolic activation) in which a pre-incubation step was added. Standard strain-specific mutagens served as positive controls. Precipitation of the test material was observed at concentrations of 2500 µg/plate and above in trial 1 (with or without metabolic activation) and at 1000 µg/plate and above in trials 2 (without metabolic activation) and 3 (with metabolic activation). No precipitation was noted at any concentration in the pre-incubation assay. No evidence of cytotoxicity was observed in any strain in the presence or absence of metabolic

activation. There were no treatment-related increases in the mean number of revertants per plate in any strain. The positive controls induced marked increases in the number of revertant colonies compared with controls in all strains in the presence and absence of metabolic activation. There was no evidence for an increased induction of mutant colonies over background (Callander, 2005).

In three independent trials of an assay for mammalian cell gene mutation at the $Tk^{+/-}$ locus, L5178Y mouse lymphoma cells cultured in vitro were exposed to mandipropamid (purity, 96.5 at concentrations of 0, 257, 515, 1030, 2060, or 4119 μg/ml (trials 1 and 2, with or without metabolic activation) or 0, 1, 10, 50, 100, 250, or 500 μg/ml (trial 3, with or without metabolic activation) in DMSO for 4 h. The study complied with OECD guideline 476. The S9 fraction used for metabolic activation was derived from the livers of male Sprague-Dawley rats induced with phenobarbital and β-naphthoflavone. Ethylmethane-sulfonate (EMS) and Benzo(α)pyrene served as positive controls in the absence and presence of metabolic activation, respectively.

Cytotoxicity (decreased mean relative survival) was observed at all doses in the presence and absence of metabolic activation in trials 1 and 2. No statistically significant or biologically relevant increases in the frequency of mutants were observed in the absence of metabolic activation. However, in the presence of metabolic activation, statistically significant increases ($p < 0.05$) in mutant frequency were noted at 2060 g/ml in trial 1 and 1030 g/ml in trial 2. These values were less than twice the values for the negative controls and were not dose-dependent, and were thus not considered to be biologically significant or treatment-related. The positive controls induced the appropriate response both in the presence and absence of metabolic activation. There was no evidence for increased induction of mutant colonies over background in the presence or absence of metabolic activation (Clay, 2002).

In two independent assays for chromosomal aberration in mammalian cells, lymphocyte cultures were prepared from human peripheral blood and exposed to mandipropamid (purity, 96.5%) at a concentration of 0, 10, 50, 100, 250, 500, 1000, 2000, 3000 or 4119 μg/ml (in the presence or absence of metabolic activation) for 3 h followed by a 17 h recovery period (trial 1); 0, 1, 2.5, 5, 10, 25, 50, 100, or 250 μg/ml for 3 h with a 17 h recovery period (in the presence of metabolic activation, trial 2); or 0, 1, 2.5, 5, 10, 25, 50, 100, or 250 μg/ml for 20 h with no recovery period (in the absence of metabolic activation, trial 2). The study complied with OECD guideline 473.

Excessive cytotoxicity was observed at concentrations of 250 μg/ml and above (in the presence or absence of metabolic activation, trial 1), 100 μg/ml and above (in the presence of metabolic activation, trial 2), and 50 μg/ml and above (in the absence of metabolic activation, trial 2). Therefore, the following concentrations were selected for evaluation of chromosomal aberrations: trial 1: 10, 50, and 100 μg/ml (in the presence or absence of metabolic activation); trial 2: 2.5, 10, and 25 μg/ml (in the absence of metabolic activation); and trial 2: 5, 25, and 50 μg/ml (in the presence of metabolic activation).

No significant increases in the mean percentage of aberrant cells were observed in either trial in the presence or absence of metabolic activation. The positive controls induced increases ($p < 0.001$) in the number of aberrant cells in the presence and absence of metabolic activation in both trials. There was no evidence of increased frequency of chromosome aberrations over background in the presence or absence of metabolic activation (Fox, 2002).

(b) In vivo

In an assay for micronucleus formation in bone marrow, groups of five male Wistar rats (age 6–7 weeks) were given a single dose of mandipropamid (purity, 96.5%) at 0 or 2000 mg/kg bw by gavage (dose volume, 10 ml/kg bw) in aqueous 0.5% methylcellulose . The study complied with OECD guideline 474. Bone-marrow cells were harvested at 24 h and 48 h after dosing.

No compound-related mortalities or clinical signs of toxicity were observed. A small decrease ($p < 0.05$) in the percentage of polychromatic erythrocytes (PCE) was observed at 48 h after dosing,

indicating that mandipropamid was slightly toxic to the bone marrow. No treatment-related increases in the frequency of micronucleated polychromatic erythrocyte (MPCE) were observed in the treated rats at either sacrifice time compared with controls. The positive control induced the appropriate response (Fox, 2005).

In an assay for unscheduled DNA synthesis assay in vivo/in vitro, rat hepatocyte cultures were prepared from groups of two to three male Wistar rats given a single oral dose of mandipropamid (purity, 96.5%) at 0 or 2000 mg/kg bw by gavage (dose volume, 10 ml/kg bw) in 0.5% carboxymethylcellulose. The study was conducted in compliance with OECD guideline 486. Hepatocytes were harvested at 2 h or 16 h after dosing.

The net nuclear grain (NNG) counts in the treated rats (–4.8 to –4.7) were well below the threshold of 0 or more NNG required for a positive response, and the mean percentage of cells in repair (> 5 NNG/cell) in the treated rats was only 1% in both trials. The positive controls induced the appropriate response in both trials. There was no evidence that unscheduled DNA synthesis, as determined by radioactive-tracer procedures (nuclear silver grain counts] was induced (Clay, 2005).

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study of reproductive toxicity, groups of 26 male and 26 female Alpk:APfSD (Wistar-derived) rats were given diets containing mandipropamid (purity, 96.5%) at a concentration of

Table 6. Results of studies of genotoxicity with mandipropamid

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation [a,b]	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2P and WP2P *uvr*A	100–5000 µg/plate ± S9, in DMSO	96.5	Negative ±S9	Callander (2005)
Forward mutation [b,c]	Mouse lymphoma cells (*Tk*$^{+/-}$ locus)	1–4119 µg/ml ±S9, in DMSO	96.5	Negative ±S9	Clay (2002)
Chromosomal aberration [d,e]	Human lymphocytes	Trial 1: 10–4119 µg/ml +S9 Trial 2: 1–250 µg/ml ±S9	96.5	Negative ±S9	Fox (2002)
In vivo					
Micronucleus formation[f,g]	Wistar rats (five males and five females per dose)	0 or 2000 mg/kg bw (single oral dose by gavage in methylcellulose)	96.5	Negative	Fox (2005)
Unscheduled DNA synthesis[f]	Rat primary hepatocytes	0 or 2000 mg/kg bw (single oral gavage dose)	96.5	Negative	Clay (2005)

DMSO, dimethyl sulfoxide; S9, 9000 × *g* supernatant from livers of male rats induced with phenobarbital and β-naphthoflavone.

[a]: Positive and negative controls included.

[b]: Tested in triplicate; positive and negative controls included.

[c] Cytotoxicity observed at all doses ± S9.

[d] Tested in duplicate; positive and negative controls included.

[e] Cytotoxicity observed at concentrations of ≥ 250 µg/ml ± S9 in trial 1 and at ≥ 100 µg/ml ± S9 in trial 2.

[f] Positive and negative controls included.

[g] Aberrations analysed in five males and five females; no clinical signs of toxicity were observed.

0, 50, 250, or 1500 ppm (equal to 0, 4.7, 22.9 or 146.3 mg/kg bw per day in males and 0, 5.0, 24.5 or 148.2 mg/kg bw per day in females, on the basis of mean pre-mating food intake of F_0 and F_1 rats). The parental (F_0) generation rats were fed the test diets for 10 weeks before mating to produce the F_1 litters. Upon weaning, F_1 parents were fed the test diets for 10 weeks before mating to produce the F_2a litters. Because of equivocal differences in live birth index, whole litter losses, and live litter size between the control and 1500 ppm groups, the F_1 parents were mated a second time to produce the F_2b litters.

There were no effects of treatment on parental mortality, clinical signs, estrus cycle duration or periodicity; sperm parameters, or gross pathology. Additionally, there were no treatment-related effects on body weights, body-weight gains, food consumption, or food utilization during gestation or lactation.

During pre-mating in the F_0 generation, food consumption was increased by 5–10% in males at 1500 ppm during weeks 1–4, while body weights were similar to those of the controls. Food utilization of these rats was decreased by 8% during weeks 1–4, resulting in a decrease (4%) in food utilization overall for the pre-mating period (weeks 1–10). At 1500 ppm in the F_1 generation, body weights were decreased by 2–8% in males throughout pre-mating, attaining significance in 7 out of 11 weeks. Weekly cumulative body-weight gains in these rats were decreased by 6–9% throughout pre-mating, resulting in a decrease of 6% in body-weight gain for the overall (weeks 1–11) pre-mating period. Food consumption in these males was decreased by 5% during weeks 9–10, and food utilization was decreased by 8% during weeks 1–4 and by 6% during weeks 5–8, resulting in a decrease of 4% for overall food utilization (weeks 1–10). At 1500 ppm, absolute and adjusted liver weights were increased in the F_0 males (17–19%) and females (7–8%) and in the F_1 males and females (11–17%). These increases attained significance ($p°\leq°0.05$) except for the absolute liver weight in the F_0 females. Because there were no microscopic findings in the liver and clinical-chemistry analyses were not performed, the increased liver weights were considered to be equivocal**.** Similar findings in the liver were noted in the short-term (Pinto, 2005a) and combined long-term/oncogenicity (Pinto, 2005b) studies in rats.

Absolute and adjusted adrenal weights were increased by 13–23% at 1500 ppm compared with those of controls in the F_0 males and F_1 males and females. Absolute adrenal weights were also increased (by 11%) in F_1 females at 250 ppm. An increase in the severity of vascular ectasia was observed in females at 1500 ppm (eight dams with minimal to moderate severity) compared with controls (seven dams with minimal severity). However, vascular ectasia was only observed in zero to four rats per group at 1500 ppm in P-generation male and females and in F_1-generation males. The sponsor stated that vascular ectasia in the adrenal is a common spontaneous age-related lesion that is seen predominantly in female rats. It was also stated that the severity and incidence observed in the F_1 dams at 1500 ppm was less than the maximum for historical controls at 1-year interim sacrifice. Although data were not available, it was stated that the incidence of this finding can be as high as 90% at 1 year and varies widely, as does severity. Therefore, the findings in the adrenal gland were considered to be equivocal.

There were no treatment-related effects on viability, clinical signs, or anogenital distance. In the F_2a litter, the live birth index was decreased at 1500 ppm (86.8%) compared with values for controls (97.7%), and the litter size at postnatal day 1 was decreased by 22% at 1500 ppm compared with values for controls. However, when the numbers of whole litter losses were excluded, these effects were not evident. At 1500 ppm, adjusted pup weights were decreased by 7–14% in male and female F_1 and F_2b pups. In the F_2a litter, pup weights of the treated males and females were comparable to those of the controls. There were no effects of treatment on total litter weight. In the F_1 parental males, the time until preputial separation was longer at 1500 ppm (44.8 days) than in the controls (43.7 days), indicating a slight delay in sexual maturation that was likely to be related to the decreased pup body weights beginning on postnatal day 15. However, the time to vaginal opening was unaffected by treatment. At 1500 ppm, relative liver weights (adjusted for body weight) were increased by 9–17%

in the F_1, F_2a, and F_2b pups. Also at this dose, absolute liver weights were increased by 14% in the F_2a females. However, because no clinical chemistry or histopathology analyses were performed, the increased liver weights were considered to be equivocal.

There were no effects of treatment on the pre-coital interval, number of females pregnant, number of complete litter resorptions, mating success, post-implantation loss, or duration of gestation in either generation. There were no effects of treatment on whole litter losses in the F_0 generation. However, in the F_1 generation, the number of whole litter losses was increased in the F_2a litter at 1500 ppm compared with controls. Therefore, the F_1 dams were mated a second time to produce the F_2b litters, and there was no effect on the number of whole litter losses, indicating that the finding in the F_2a litter was likely to be incidental.

The NOAEL for parental toxicity was 250 ppm (equivalent to 22.9 mg/kg bw per day in males and 24.5 mg/kg per day in females) on the basis of decreased body weights, body weight gains, food consumption, and food efficiency in males. The NOAEL for reproductive toxicity was 1500 ppm (equivalent to 146.3 mg/kg bw per day in males and 148.2 mg/kg bw per day in females) as no LOAEL was identified. The NOAEL for offspring toxicity was 250 ppm (equivalent to 22.9 mg/kg bw per day in males and 24.5 mg/kg per day in females), on the basis of decreased pup weights at 1500 ppm (Milburn, 2005b).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 24 time-mated female A1pk:AP$_f$SD (Wistar-derived) rats were given mandipropamid, (purity, 96.5%) at a dose of 0, 50, 200, or 1000 mg/kg bw per day by oral gavage in 0.5% aqueous carboxymethylcellulose in a treatment volume of 1 ml/100 g bw from day 5 until day 21 of gestation. All dams were killed on day 22 of gestation; fetuses were removed by cesarean section and examined.

All females survived to scheduled sacrifice, and there were no clinical signs of toxicity. There were no treatment-related, adverse effects on body weight, body-weight gains, food consumption, clinical chemistry, liver weights, or gross pathology at any dose.

There were no abortions, premature deliveries, or complete litter resorptions and no effects of treatment on the numbers of litters, live fetuses, dead fetuses, resorptions (early or late) or on fetal sex ratio, or post-implantation loss, fetal body weights, gravid uterine weights, or skeletal ossification in the fetuses. There was no treatment-related effect on the ossification scores of the paws. There were no treatment-related external, visceral, or skeletal variations or malformations.

The NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Moxon, 2005a).

Rabbits

In a study of developmental toxicity, 24 time-mated female New Zealand White rabbits were given mandipropamid (purity, 96.5%) at a dose of 0, 50, 250, or 1000 mg/kg bw per day by oral gavage in 0.5% aqueous carboxymethylcellulose in a treatment volume of 4 ml/kg bw from day 5 until day 29 of gestation. All surviving rabbits were killed on day 30 of gestation; fetuses were removed by caesarean section and examined.

There were no treatment-related mortalities and no adverse clinical signs of toxicity. There were no treatment-related, adverse effects on body weight, body-weight gains, food consumption, or gross pathology at any dose.

At 250 mg/kg bw per day, one female aborted; otherwise, there were no premature deliveries or complete litter resorptions and no effects of treatment on the numbers of litters, live fetuses, dead

fetuses, resorptions (early or late) or on fetal body weights, sex ratio, or post-implantation loss, fetal body weights, gravid uterine weights, or skeletal ossification in the fetuses. There were no treatment-related external, visceral, or skeletal variations or malformations. There was no treatment-related effect on the ossification scores of the paws.

The NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Moxon, 2005b).

2.6 Special studies

(a) Acute neurotoxicity

In a study of acute neurotoxicity, groups of 10 male and 10 female fasted Alpk:AP$_f$SD (Wistar-derived) rats were given mandipropamid (purity, 96.5%) as a single dose at 0, 200, 600, or 2000 mg/kg bw by gavage in 0.5% carboxymethylcellulose (dose volume, 10 ml/kg bw) and were sacrificed on day 15 of the study. FOB and motor activity testing were performed on all rats before exposure, and on days 1 (at 2–4 h after dosing), 8, and 15. At study termination, five rabbits per group were anaesthetized and perfused in situ for neuropathological examination. The brain and peripheral nervous system tissues collected from the perfused animals in the control group and the group at 2000 mg/kg bw were subjected to histopathological evaluation.

No treatment-related effects were observed on mortality, clinical signs, body weight, body-weight gains, food consumption, FOB parameters, motor activity, or neuropathology.

The NOAEL was 2000 mg/kg bw, the highest dose tested (Milburn, 2005c).

(b) Short-term study of neurotoxicity

In a short-term study of neurotoxicity, groups of 12 male and 12 female Alpk:AP$_f$SD rats were given diets containing mandipropamid (purity, 96.5%) at a concentration of 0, 100, 500 or 2500 ppm (equal to 0, 7.4, 37.3 or 192.5 mg/kg bw per day in males and 0, 8.4, 41.0 or 206.7 mg/kg bw per day in females) for at least 90 consecutive days. Neurobehavioral assessment (FOB and motor activity testing) was performed in all rats at weeks –1, 2, 5, 9, and 14. At study termination, five males and five females per group were anaesthetized and perfused in situ for neuropathological examination. The tissues from the perfused animals in the control group and in the group at 2500 ppm were subjected to histopathological evaluation.

No adverse, treatment-related effects were observed on mortality, clinical signs, food consumption, functional observational battery parameters, motor activity, brain weights, or neuropathology. In males at 2500 ppm, body weights were decreased by 3% initially at week 1 and by 2–7% throughout the study, and decreased cumulative body weight (10–11%) was observed from week 10 until termination. Decreased food utilization (16%) was noted during weeks 5–8. Decreases were also observed during weeks 1–4 (6%) and weeks 9–13 (12%), leading to a decrease in overall food utilization in weeks 1–13 (9%). The effect on food utilization was of a similar magnitude to that observed on body weight and body-weight gain. There were no effects on body weight, body-weight gain, food consumption or food utilization in females. Slight increases of 13–26% were observed at 2500 ppm adjusted liver weight after perfusion in females, and in absolute and adjusted liver weights (without perfusion) in males and females. The effect on the liver was considered equivocal in the absence of any other evidence supporting hepatotoxicity. No neurological effects were observed at any dose in males or females.

The NOAEL was 500 ppm, equal to 37 mg/kg bw per day in males and 41 mg/kg bw per day in females**,** as no LOAEL was identified (Pinto, 2005c).

3. Observations in humans

No information was available.

Comments

Biochemical aspects

The extent of absorption of radiolabelled mandipropamid was similar in male and female rats dosed by gavage. Absorption was incomplete, with 67–74% of the administered dose being absorbed at the lower dose (3 mg/kg bw) and only 30–45% absorbed at the higher dose (300 mg/kg bw). Absorption was more rapid in females, with peak blood concentrations occurring at 4.5 h for the lower dose and 10 h at the higher dose, while for males the values were 8.5 h and 24 h, respectively. Little or no radioactivity was recovered in the expired air (less than 0.16% of the administered dose). Excretion was predominantly via the bile (lower dose) and faeces and > 90% of the administered dose was eliminated within 168 h. In males, a greater proportion of the administered dose was excreted in the faeces, while in females a significantly greater proportion was excreted via the urine. The greater extent of biliary elimination in males than in females was consistent with faecal excretion being the major route of elimination in males (73%) and of lesser importance in females (55%) at the lower dose. Some reabsorption of biliary metabolites was apparent at both doses. Tissue retention of the radiolabelled material was low, even after multiple doses. The total concentration of tissue residues including the carcass was < 0.3%, therefore demonstrating no evidence of bioaccumulation. The highest concentration of residues was found in the liver.

The biotransformation of mandipropamid was relatively simple since no cleavage of the molecule was observed. The major metabolic transformations involved loss of one or both of the propargyl groups of the molecule, followed by glucuronidation and *O*-demethylation, to produce six major metabolites. While the qualitative metabolite profile was largely independent of sex and dose, quantitative differences were found. Increasing the dose resulted in increasing amounts of radioactivity isolated as parent, indicating saturation of metabolic processes.

Toxicological data

Mandipropamid was of low acute toxicity in rats given a single dose orally (LD_{50} > 5000 mg/kg bw), dermally (LD_{50} > 5000 mg/kg bw) or by inhalation (LC_{50} > 5.19 mg/l). Mandipropamid was minimally irritating to the skin and eyes and was not found to be a dermal sensitizer (local lymph node assay in mice).

In short-term studies of toxicity with mandipropamid, the target organ was the liver, at doses that also resulted in decreased body weight and body-weight gain.

In a 90-day dietary study in rats, decreased body weight, body-weight gain and food consumption were observed at doses of 3000 ppm (260 mg/kg bw per day) and above. Various erythrocyte parameters (haemoglobin, erythrocyte volume fraction, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration) were decreased in males and females at doses of 3000 ppm (260 mg/kg bw per day) and above. Increases in liver weight (both sexes), plasma gamma-glutamyl transferase (58–105% in females only) at doses of 3000 ppm (260 mg/kg bw per day) and above were not considered to be adverse in the absence of findings of liver toxicity. The periportal hypertrophy/eosinophilia observed in the liver was considered to be treatment-related, but of questionable toxicological significance given the minimal to slight severity and the lack of any evidence of progression in the long-term study. The NOAEL for toxicity in rats was 500 ppm (41 mg/kg bw per day).

In dogs given capsules containing mandipropamid, increases in liver weights, cholesterol concentration, alkaline phosphatase and ALT activity were seen after 13 weeks at 100 mg/kg bw per day.

The NOAEL in the 90-day study in dogs was 25 mg/kg bw per day. In the 1-year study in dogs, body-weight gain and food consumption were decreased at 400 mg/kg bw per day, together with increased liver weight. At 40 mg/kg bw per day or above, there was also increased alkaline phosphatase and ALT activity and minimal to moderate pigmentation of the liver by porphyrin. The NOAEL in the 1-year study in dogs was 5 mg/kg bw per day. Considering the spacing of doses used and on the basis of the similarity of effects observed in the 90-day and 1-year studies in dogs, the overall NOAEL for dogs was 25 mg/kg bw per day.

In an 80-week study, mice were given diets containing mandipropamid at a concentration of 0, 100, 500 or 2000 ppm, equal to 0, 11, 55 or 223 mg/kg bw per day. There were no treatment-related changes in survival, or the incidence of tumours or non-neoplastic lesions. The only significant findings were observed at the highest dose: reductions in body weight and food conversion efficiency. There was no evidence of carcinogenicity with mandipropamid in this study. The NOAEL was 55 mg/kg bw per day.

In a 2-year (104-week) study, rats were given diets containing mandipropamid at a concentration of 0, 50, 250 or 1000 ppm, equal to 0, 3, 15 or 61 mg/kg bw per day. There were no treatment-related changes in survival or the incidence of tumours. The only significant findings were in males at the highest dose: reductions in body weight, body-weight gain and food conversion efficiency, gross and histopathological changes in the kidneys (roughened surface, and increased severity of chronic progressive nephropathy), and associated osteodystrophia fibrosa and histopathological changes in the parathyroid (increased severity of hyperplasia). Mandipropamid was not carcinogenic in this study. The NOAEL was 15 mg/kg bw per day.

Mandipropamid gave negative results in an adequate range of studies of genotoxicity in vitro and in vivo. The Meeting concluded that mandipropamid was unlikely to be genotoxic.

On the basis of the absence of carcinogenicity in rodents and the absence of genotoxicity, the Meeting concluded that mandipropamid is unlikely to pose a carcinogenic risk to humans.

In a multigeneration study of reproductive toxicity in rats, the target organ was also the liver. No reproductive effects were observed. The NOAEL for parental systemic toxicity was 250 ppm, equal to 22.9 mg/kg bw per day, on the basis of slightly lower body weight and body-weight gain in F_1 males during premating and increased absolute and relative liver weights in male and female parental animals sexes and in F_1 females. The NOAEL for reproductive toxicity was 1500 ppm, equal to 146.3 mg/kg bw per day, the highest dose tested. Toxicity observed in offspring at 1500 ppm included decreased pup weight from day 15 of lactation, increased liver weights in both generations and an increased time to preputial separation in male F_1 pups. The NOAEL for offspring toxicity was 250 ppm, equal to 22.9 mg/kg bw per day.

In a study of developmental toxicity in rats, the NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested. No developmental toxicity or teratogenicity was observed. In rabbits, no effects were observed in dams or fetuses at up to the limit dose of 1000 mg/kg bw per day.

In a study of acute neurotoxicity in rats, mandipropamid exhibited no systemic toxicity or evidence of neurotoxicity at 2000 mg/kg bw. In a 13-week study of neurotoxicity in rats, systemic toxicity was observed at 2500 ppm, equal to 192 mg/kg bw per day, as reductions in body weight, body-weight gain and food efficiency. No evidence of neurotoxicity was observed. The NOAEL was 37 mg/kg bw per day.

There were no reports of adverse health effects in manufacturing-plant personnel or in operators and workers exposed to mandipropamid formulations.

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

Toxicological evaluation

The Meeting established an actable daily intake (ADI) of 0–0.2 mg/kg bw based on the NOAEL of 15.2 mg/kg bw per day, identified on the basis of decreased body weight and kidney effects (increased severity of chronic progressive nephropathy and associated osteodystrophia fibrosa) at 61.3 mg/kg bw per day in the long-term dietary study in rats and using a safety factor of 100.

The Meeting noted that mandipropamid was not acutely toxic after short-term dosing, that there were no adverse findings in a study of acute neurotoxicity and that mandipropamid did not exhibit developmental toxicity. The Meeting concluded that the establishment of an acute reference dose (ARfD) was unnecessary.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year studies of toxicity and carcinogenicity [a]	Toxicity	500 ppm, equal to 55 mg/kg bw per day	2000 ppm, equal to 223 mg/kg bw per day
		Carcinogenicity[d]	2000 ppm, equal to 223 mg/kg bw per day	—
Rat	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	250 ppm, equal to 15 mg/kg bw per day	1000 ppm, equal to 61 mg/kg bw per day
		Carcinogenicity[d]	1000 ppm, equal to 61 mg/kg bw per day	—
	Multigeneration study of reproductive toxicity[a]	Parental toxicity	250 ppm, equal to 23 mg/kg bw per day	1500 ppm, equal to 146 mg/kg bw per day
		Offspring toxicity	250 ppm, equal to 23 mg/kg bw per day	1500 ppm, equal to 146 mg/kg bw per day
		Reproduction[d]	1500 ppm, equal to 146 mg/kg bw per day	—
	Developmental toxicity[a,b]	Maternal toxicity[d]	1000 mg/kg bw per day	—
		Embryo and fetal toxicity[d]	1000 mg/kg bw per day	—
Rabbit	Developmental toxicity [,b]	Maternal toxicity[d]	1000 mg/kg bw per day	—
		Embryo and fetal toxicity[d]	1000 mg/kg bw per day	—
Dog	90-day and 1-year study of toxicity [c]	Toxicity	25 mg/kg bw per day [e]	40 mg/kg bw per day

[a] Dietary administration.
[b] Gavage administration.
[c] Capsule administration.
[d] Highest dose tested.
[e] Based on an overall NOAEL from the two studies.

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 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 mandipropamid

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid, extent dependent on dose, 67–74% at the lower dose, 30–45% at the higher dose
Distribution	Highest concentrations in the liver and kidney
Potential for accumulation	No evidence
Rate and extent of excretion	High, virtually complete by 168 h
Metabolism in animals	Mainly glucuronidation (> 50% of excreted dose mandipropamid glucuronide)
Toxicologically significant compounds (animals, plants and environment)	Parent
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 5050 mg/kg bw
Rat, LC_{50}, inhalation	> 5.19 mg/l air
Rabbit, dermal irritation	Minimal irritation
Rabbit, ocular irritation	Minimal irritation
Mouse, dermal sensitization	Not sensitizing (local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Liver, body weight
Lowest relevant oral NOAEL	25 mg/kg bw per day (90-day and 1-year study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (28-day study in rats, highest dose tested)
Lowest relevant inhalation NOAEL	No data
Genotoxicity	
	Not genotoxic

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Body weight, kidney, parathyroid
Lowest relevant NOAEL	15 mg/kg bw per day (rats)
Carcinogenicity	Not carcinogenic in rats and mice

Reproductive toxicity

Reproduction target/critical effect	None
Lowest relevant reproductive NOAEL	146 mg/kg bw per day (rats, highest dose tested)
Developmental target/critical effect	None
Lowest relevant developmental NOAEL	1000 mg/kg bw per day (rats, rabbits, highest dose tested)

Neurotoxicity/delayed neurotoxicity

Acute neurotoxicity and studies of short-term neurotoxicity	No indications of neurotoxicity in studies of acute toxicity or repeat-dose studies

Medical data

	No occupational or accidental poisoning reported

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.2	Rat, 2-year study	100
ARfD	Unnecessary	—	—

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PROTHIOCONAZOLE and PROTHIOCONAZOLE-DESTHIO

First draft prepared by
D.B. McGregor[1] and Roland Solecki[2]

[1]Toxicity Evaluation Consultants, Aberdour, Scotland; and
[2]Federal Institute for Risk Assessment, Berlin, Germany

Explanation

Prothioconazole is the ISO approved common name for the substance for which the IUPAC nomenclature is 2-[(2*RS*)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2*H*-1,2,4-triazole-3(4*H*)-thione (CAS No. 178928-70-6). It is a systemic triazolinthione fungicide, the targets for which are most of the economically important diseases caused by *Ascomycetes*, *Basidiomycetes* and *Deuteromycetes* in cereals, oilseed rape and peanuts. Its mode of action is interference with the synthesis of ergosterol in the target fungi by inhibition of CYP51, which catalyses demethylation at C14 of lanosterol or 24-methylene dihydrolanosterol, leading to morphological and functional changes in the fungal cell membrane.

The residue definition for risk assessment in plant commodities is the metabolite prothioconazole-desthio, [CAS name 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-

dihydro-3*H*-1,2,4-triazole], while in animal commodities it is the sum of prothioconazole-desthio, prothioconazole-desthio-3-hydroxy (M14) and prothioconazole-desthio-4-hydroxy (M15), and their conjugates expressed as prothioconazole-desthio. For the active ingredient prothioconazole and its metabolite prothioconazole-desthio, complete data sets were submitted. Like the parent compound, prothioconazole-desthio has fungicidal properties as a consequence of being an inhibitor of ergosterol biosynthesis, although it has never been fully developed as an active ingredient.

While no independent studies of toxicity with M14 and M15 were available, both metabolites and their glucuronide conjugates were identified and quantified in studies with prothioconazole and prothioconazole-desthio in rats; the toxicology of M14 and M15 can thus be considered to be included in the databases provided for these compounds.

Prothioconazole 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

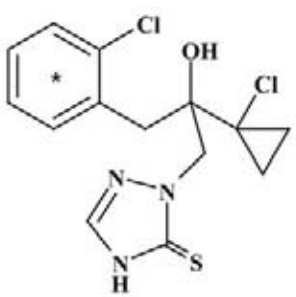
Prothioconazole

1. Biochemical aspects: absorption, distribution, and excretion

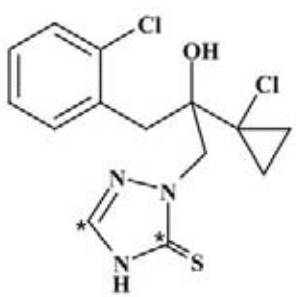
The absorption, distribution and excretion characteristics of prothioconazole were studied extensively in male and female Wistar rats (Justus, 2001a). The study was conducted using [triazole-UL-^{14}C]- and [phenyl-UL-^{14}C]-labelled prothioconazole. The structures and radiolabel positions are shown in Figure 1.

Figure 1. Chemical structures of radiolabelled prothioconazole used in a study of absorption, distribution and excretion

[Phenyl-UL-^{14}C]prothioconazole



[Triazole-UL-^{14}C]prothioconazole



* = position of radiolabel

The chemical and radiochemical purities of these compounds were as follows: [phenyl-UL-^{14}C]rothioconazole: chemical purity, > 99% for four batches and > 98% for one batch, radiochemical purity, > 99% for four batches and > 98% for one batch; [triazole-UL-^{14}C]prothioconazole: chemical purity, > 99%, radiochemical purity, > 99%. The rats received single or repeated high or low doses by gavage (except for group 4 which was treated by intraduodenal administration) according to the study design outlined in Table 1. The balances of recovered radioactivity after these various treatments are presented in Table 2.

Table 1. Outline of the design of a study of absorption, distribution and excretion of radiolabelled prothioconazole in rats

Group No.	Treatment	Dose	Route	Radiolabel (^{14}C)	No/sex	Duration	Fluids/tissues sampled
1 and 2	Single low dose	2 mg/kg bw	Oral	Triazole-UL	Five males and five females	7 days	Urine, faeces, organs, carcass, skin, gastrointestinal tract
3	Single high dose	150 mg/kg bw	Oral	Triazole-UL	Five males	7 days	Urine, faeces, organs, carcass, skin, gastrointestinal tract
4[a]	Single low dose	2 mg/kg bw	Duodenal (bile-duct cannulation)	Triazole-UL	Eight males	48 h	Urine, faeces, bile, carcass, skin, gastrointestinal tract
8	Single low dose, expired air test	2 mg/kg bw	Oral	Phenyl-UL	Five males	48 h	Expired air, urine, faeces, carcass, skin, gastrointestinal tract
9	Single low dose, EPA basic test	5 mg/kg bw	Oral	Phenyl-UL	Five males	48 h	Urine, faeces, organs, carcass, skin, gastrointestinal tract
11[a]	Single low dose	2 mg/kg bw (bile-duct cannulation)	Oral	Phenyl-UL	Twenty males	6 h	Urine, faeces, bile, carcass, skin, gastrointestinal tract
12	Multiple low doses	2 mg/kg × 15[b]	Oral	Phenyl-UL	Five males	48 h	Urine, faeces, organs, carcass, skin, gastrointestinal tract
16	Single high dose	150 mg/kg bw	Oral	Triazole-UL	Five females	7 days	Urine, faeces, organs, carcass, skin, gastrointestinal tract
18	Multiple low doses	2 mg/kg bw × 16[c]	Oral	Phenyl-UL	Five females	48 h	Urine, faeces, organs, carcass, skin, gastrointestinal tract

From Justus (2001a)

EPA, Environmental Protection Agency

[a] Animals surgically prepared with biliary and duodenal cannulae 24 h before treatment.

[b] Fourteen unlabelled doses followed by one labelled dose.

[c] Fifteen unlabelled doses followed by one labelled dose.

After an oral dose, prothioconazole was rapidly and extensively absorbed, the T_{max} calculated from plasma concentrations being < 1 h (Table 5). Of the administered dose, 90% was excreted via the bile (Table 2). Bile-duct cannulated rats receiving phenyl-labelled material were killed after 6 h, the main intention being to collect bile for metabolite identification rather than for kinetic studies; however, comparison with the equivalent experiment with the triazole label (group 4) suggests that excretion over 48 h would exceed 90%. Although this is an extrapolation, it was considered to be acceptable owing to similarities in the metabolism of the differently radiolabelled compounds.

Table 2. Balance of radioactivity at the end of each study (percentage of the administered dose)

Tissue/organ	Group (as in Table 1)									
	1	2	3	4	8	9	11	12	16	18
Expired air	—	—	—	—	0.062	—	—	—	—	—
Urine	10.47	15.97	3.710	2.048	5.899	4.569	1.154	5.137	11.81	10.24
Bile	—	—	—	90.21	—	—	82.17	—	—	—
Faeces	84.49	78.40	95.88	1.28	80.59	85.37	1.524	93.22	87.76	86.80
Skin	0.08	0.06	< LOD	0.12	0.07	0.05	0.19	0.05	< LOD	0.02
Sum of organs	1.34	0.27	0.09	0.72	2.17	2.63	3.01	2.85	0.09	0.35
Body excluding gastrointestinal tract	1.41	0.32	0.09	0.840	2.24	2.68	3.20	2.90	0.09	0.37
Gastrointestinal tract	0.13	0.07	0.02	0.06	1.34	3.14	19.62	0.92	0.02	0.46
Total body	1.54	0.39	0.11	0.90	3.57	5.82	22.81	3.82	0.11	0.83
Balance	96.50	94.76	99.70	94.14	90.13	95.76	107.70	102.20	99.68	97.88

From Justus (2001a)

LOD, limit of detection; —, not determined.

Table 3. Recovery of radioactive residues in organs and tissues at sacrifice of rats given radiolabelled prothioconazole)

Organ/tissue	Recovery of radiolabel (% of administred dose) (as in Table 1)						
	1 (168 h)	2 (168 h)	3 (168 h)	9 (48 h)	12 (48 h)	16 (168 h)	18 (48 h)
Gastrointestinal tract	0.13	0.07	0.02	3.14	0.92	0.02	0.46
Liver	1.13	0.05	0.08	2.23	2.62	0.01	0.11
Kidneys	0.02	0.01	0.002	0.030	0.03	0.002	0.012
Skin	0.07	0.06	< LOD	0.05	0.05	< LOD	0.02
Body excluding gastrointestinal tract	1.41	0.32	0.09	2.68	2.90	0.09	0.37
Total body	1.54	0.39	0.11	5.82	3.82	0.11	0.83

From Justus (2001a)

< LOD, less than the limit of detection.

The concentrations of radiolabel in various organs are shown in Table 3. Concentrations of radiolabel levels in the liver were markedly (23-fold) higher in males than in females given single or repeated doses of either radiolabel at 2 mg/kg bw. The difference between males and females was lower (sixfold) after a single dose at 150 mg/kg bw. There were no other notable differences between the sexes or between rats receiving the higher or lower doses. The only notable effect of repeated doses compared with single doses was higher concentrations of radiolabel in the gastrointestinal tract after repeated doses.

Dose-normalized concentrations were < 0.02 mg/kg in all tissues except the liver, kidney, gastrointestinal tract and thyroid. In terms of parent-compound equivalents, the concentrations in the thyroid were highest (up to 9.5 mg/kg after a dose of 150 mg/kg bw), but in many study groups,

concentrations in the thyroid were below the limit of detection, which is relatively high in the thyroid owing to its small size.

Only 0.06% of the administered dose was eliminated via expired air (group 8 in Table 2), so this route of excretion received no further consideration (Justus, 2001a).

Whole-body audioradiography

In s astudy of whole-body autoradiography, 1 h after oral administration, the most intense signal was detected in the small intestine, stomach, oesophagus and liver, followed by the kidney in males and females. Radiolabel was also detected in the urinary bladder in males, but not in females. All other organs and tissues showed markedly less blackening. The pattern of distribution of radiolabel in the small intestine and also in the gastric mucosa of the stomach indicated that extrabiliary secretion into the gastrointestinal tract had occurred; this effect was more pronounced in females.

Quantitative whole-body autoradiography showed, in general, a continuous decrease in the concentration of prothioconazole equivalents in most organs and tissues in males between 1 and 168 h after treatment (Table 4). However, the concentration of equivalents in most organs and tissues in females reached peak values at 8 h after treatment and thereafter showed a continuous decrease until 168 h after treatment. This indicated a moderate delay in absorption for females compared with males.

The highest concentrations of radiolabel occurred in the liver (up to 1.78 µg/g tissue in males and up to 0.97 µg/g tissue in females). Lower concentrations of equivalents occurred in the renal medulla and urinary bladder (up to 0.64 µg/g tissue) and in the brown and perirenal fat, thyroid and adrenal gland (up to 0.4 µg/g tissue). All other organs and tissues showed maximum values that were less than 0.13 µg/g tissue. In all organs and tissues, the concentrations of equivalents decreased by several orders of magnitude during the test period.

At 168 h after treatment, the concentration of equivalents in most organs and tissues was below the LOD or below the limit of quantification (LOQ). Low values, just above the limit of quantification (0.005–0.02 µg/g tissue) occurred in the renal cortex and renal medulla, blood (males only) and in the thyroid gland (females only) at this time. The concentration in the liver of male rats had decreased to 0.17 µg/g tissue, while the concentration in the liver of females was below the LOQ. The results indicated continuous elimination of radioactivity from the organs and tissues.

Table 4. Total concentrations of radioactive residues in organs and tissues of rats given radiolabelled prothioconazole in a study of whole-body radiography

Organ/tissue	Total concentration of radioactive residues (µg active substance equivalent/g wet weight)							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Males								
Blood	0.106	0.082	0.056	0.037	0.031	0.015	0.015	0.013
Liver	1.778	1.575	0.758	0.537	0.682	0.194	0.242	0.165
Renal cortex	0.300	0.179	0.115	0.061	0.050	0.023	0.020	0.018
Renal medulla	0.644	0.392	0.229	0.109	0.081	0.037	0.028	0.019
Brown fat	0.355	0.134	0.104	0.044	< LOQ[a]	< LOD[b]	< LOD[b]	—
Perirenal fat	0.285	0.117	0.083	< LOQ[a]	< LOD	< LOD	< LOD	< LOD
Urinary bladder	0.114	0.146	—	—	—	—	—	—
Muscle	0.031	0.019	0.016	0.010	< LOQ[a]	< LOD	< LOD	< LOD
Heart	0.053	0.033	0.027	0.012	< LOQ[a]	< LOD	< LOD	< LOD
Lung	0.091	0.052	0.029	0.023	0.008	0.010	0.008	0.006[a]
Spleen	0.032	0.028	0.021	0.016	0.011	< LOQ[a]	< LOQ[a]	< LOQ[a]

Pancreas	0.079	0.042	0.027	0.014	0.011	< LOQ[a]	< LOD	< LOD
Bone	< LOQ[a]	—	—	—	—	—	—	—
Bone marrow	0.030	0.033	—	0.020	0.010[a]	< LOD	—	—
Testes	0.029	0.012	0.011	0.006	0.003[a]	< LOD	< LOD	< LOD
Brain	0.022	0.012	< LOQ[a]	<LOQ[a]	< LOD	< LOD	< LOD	< LOD
Spinal cord	0.030	0.012	< LOQ[a]	<LOQ[a]	< LOD	< LOD	< LOD	< LOD
Pituitary gland	0.052	0.032	0.025	0.013	0.011	<LOQ[a]	<LOQ[a]	—
Pineal body	—	—	0.038	0.015	0.007[a]	< LOD	<LOQ[a]	—
Adrenal gland	0.265	0.128	0.097	0.041	0.030	0.014	<LOQ[a]	<LOQ[a]
Thymus	0.027	0.019	0.015	0.010	<LOQ[a]	< LOD	< LOD	< LOD
Thyroid gland	0.226	0.077	0.128	0.042	0.026	0.020	<LOQ[a]	<LOQ[a]
Salivary gland	0.062	0.040	0.028	0.014	<LOQ[a]	< LOD	< LOD	< LOD
Nasal mucosa	0.102	0.052	0.030	0.017	0.015	<LOQ[a]	<LOQ[a]	< LOD
Skin	0.103	0.067	0.059	0.060	0.041	0.031	0.033	<LOQ[a]
Eye	0.012	0.010	<LOQ[a]	<LOQ[a]	<LOQ[a]	< LOD	< LOD	< LOD
Females								
Blood	0.118	0.079	0.125	0.025	0.024	0.024	< LOQ[a]	< LOQ[a]
Liver	0.973	0.551	0.855	0.064	0.049	0.049	0.020	< LOQ[a]
Renal cortex	0.110	0.072	0.118	0.022	0.025	0.034	0.012	0.006
Renal medulla	0.152	0.121	0.207	0.025	0.025	0.028	0.011	0.007
Brown fat	0.246	0.131	0.247	0.045	< LOQ[a]	0.038	—	—
Perirenal fat	0.093	0.095	0.134	< LOD[b]	< LOD	< LOD	< LOD	< LOD
Urinary bladder	—	—	0.626	—	0.014	0.007	—	< LOD
Muscle	0.022	0.015	0.021	< LOQ[a]	< LOQ[a]	< LOQ[a]	< LOD	< LOD
Heart	0.069	0.035	0.063	0.009	0.008	0.009	<LOQ[a]	< LOD
Lung	0.081	0.052	0.052	0.017	0.012	0.016	0.008	< LOQ[a]
Spleen	0.032	0.024	0.041	0.012	0.012	0.012	< LOQ[a]	< LOQ[a]
Pancreas	0.051	0.048	0.066	0.012	0.011	0.012	< LOD	< LOD
Bone	0.023[a]	0.028[a]	—	—	—	—	—	—
Bone marrow	0.031	0.028	0.060	0.020	0.016	0.013	< LOQ[a]	—
Ovaries	0.047	0.046	0.068	0.012	0.010	0.011	< LOQ[a]	< LOQ[a]
Uterus	0.063	—	0.101	0.019	0.018	0.012	< LOQ[a]	< LOD
Brain	0.023	0.012	0.019	< LOD	< LOD	< LOD	< LOD	< LOD
Spinal cord	0.025	0.014	0.022	< LOD	< LOD	< LOD	< LOD	< LOD
Pituitary gland	0.059	0.040	0.060	0.013	0.011	0.011	< LOD	—
Pineal body	0.043	0.019	0.014	—	0.011	0.011	< LOD	—
Adrenal gland	0.142	0.090	0.142	0.028	0.025	0.023	< LOQ[a]	< LOQ[a]
Thymus	0.021	0.016	0.032	0.010	0.012	0.011	< LOQ[a]	< LOD
Thyroid gland	0.228	0.186	0.287	0.075	0.102	0.059	0.036	0.018
Salivary gland	0.063	0.042	0.079	0.014	0.013	0.013	< LOQ[a]	< LOD
Nasal mucosa	0.050	0.036	0.066	0.010	0.011	0.008[a]	< LOQ[a]	< LOD
Skin	0.060	0.055	0.084	0.033	0.023	0.023	< LOQ[a]	< LOD
Eye	0.008	< LOQ[a]	0.011	< LOD	< LOD	< LOD	< LOD	< LOD

From Justus (2001b)

[a] Less than the limit of quantitation (< LOQ)

[b] Less than the limit of detection (< LOD)

LOD, limit of detection; LOQ, limit of quantitation; —, not found in sections.

The results of this study (Justus, 2001b) are broadly consistent with those of the study of absorption, distribution and excretion described above (Justus, 2001a) in showing that prothioconazole is rapidly absorbed after oral dosing and then extensively excreted, mainly via the faeces. The highest concentrations of material were recorded in the stomach, small and large intestines, liver, kidney and urinary bladder. Moderate concentrations were also recorded in the brown and perirenal fat and in the adrenal and thyroid glands. Lower concentrations were detected in all other organs and tissues analysed. The previous study (Justus, 2001a) using dissection and liquid-scintillation counting (LSC) methods had indicated that high concentrations of radiolabel were located in the thyroid. The present study showed moderate concentrations in the thyroid, not the highest of the tissues and organs analysed but higher than most. Since the lowest accuracy with dissection and LSC is obtained when applied to the smallest tissues and organs, the results of the QWBA were considered to be more reliable.

The results show an early peak of radiolabel in tissues and organs, followed by a continuous decline by several orders of magnitude. Relative to males, female rats showed a moderately delayed absorption, markedly lower concentrations in the liver, higher concentrations in the thyroid gland and a more pronounced extrabiliary secretion into the gastrointestinal tract. Thee sex-dependent findings of the present study (Justus, 2001b) are also consistent with the results of the previous study (Justus, 2001a).

Kinetics

The plasma concentration data and excreta data from this study (Justus, 2001a) showed that the majority of the administered radioactivity was eliminated within 24 h (two elimination half-lives were indicated). Over the first 24 h, the plasma concentration-with-time data were comparable between high and low doses and with multiple low-dose treatments. Minor differences between groups after 24 h were not considered to be significant because concentrations were low by this time, most of the dose having already been eliminated. The oscillations in the plasma radioactivity concentrations with time (most pronounced in the tests with female animals) were attributed to enterohepatic recirculation, and this affected the calculated pharmacokinetic parameters (Table 5), particularly the large variations in the C_{max} values. There was no obvious effect of multiple dosing on pharmacokinetic parameters.

The cumulative excretion of radioactivity from the different groups clearly showed that urine is a minor route of excretion compared with faeces. The results from the bile-duct cannulated rats show that most of the administered dose is excreted via bile within a few hours after dosing. Comparison of the two groups of bile-duct cannulated rats (oral administration and intraduodenal administration) indicates a slightly delayed excretion after oral administration which presumably represents the oral absorption phase. The results from the bile-duct cannulated rats suggest that the material being excreted in the faeces (after dosing at 2 mg/kg bw) had previously been systemically absorbed and then excreted via the bile. Most (generally > 70%) of the administered dose was excreted in the first 24 h with only a low rate of excretion thereafter.

The slowest excretion was seen in females receiving a single lower dose (group 2 in Table 2). The delayed excretion via faeces in group 2 combined with a greater extent of excretion via urine in this group (relative to the equivalent group of males, i.e. group 1) would be consistent with enterohepatic recirculation taking place to a greater extent in females than in males. This is consistent with the temporal oscillations seen in the plasma concentrations of radiolabel recorded in group 2, mentioned above. The results from the groups receiving a single higher dose also show a greater extent of urinary excretion in females and a slightly delayed excretion via the faeces in females. Although no bile data were available in rats receiving the higher dose, this pattern of excretion was similar to that in rats receiving the lower dose and suggested that substantial systemic absorption and subsequent biliary excretion and enterohepatic recirculation also occurred at the higher dose. The lower level of

Table 5. Pharmacokinetic parameters in rats given radiolabelled prothiaconazole

Parameter	Group[a]						
	1	2	3	9	12	16	18
AUC (µg/ml × h)	6.31	8.43	358	5.84	1.77	249	1.67
$T_{1/2\ a}$ (h)	0.172	0.233	0.180	0.056	0.078	0.082	0.011
$T_{1/2\ e(1)}$ (h)	0.926	0.499	0.404	0.446	0.597	0.350	0.424
$T_{1/2\ e(2)}$ (h)	16.8	18.7	9.83	8.08	11.9	9.16	8.91
$T_{lag\ a}$ (h)	0.034	0.133	0.065	0.052	0.025	0.046	0.001
Cl (ml/min per kg)	5.28	3.96	6.99	14.30	18.83	10.00	19.90
Cl_R (ml/min per kg)	0.57	0.67	0.26	0.68	0.95	1.19	2.08
C_{max} (µg/ml)	0.43	0.92	69.80	0.65	0.47	45.00	0.35
T_{max} (h)	0.43	0.52	0.71	0.18	0.21	0.63	0.38
C_{max}[exp] (µg/ml)	0.42	1.96	71.92	0.65	0.45	41.80	0.34
T_{max}[exp] (h)	0.33	0.08	0.66	0.16	0.16	0.66	0.16
MRT (h)	23.5	25.3	11.3	11.7	15.3	11.3	10.1
MRT_{abs} (h)	0.28	0.47	0.65	0.13	0.31	0.55	0.61
MRT_{disp} (h)	23.2	24.9	10.7	11.5	15.0	10.7	9.5

From Justus (2001a)

abs, absorption; AUC, area under the curve of concentration–time; Cl, clearance; MRT, mean residence time of the radioactivity or total radioactivity in the measurement compartments; MRTabs, mean residence time of the absorption; MRTdisp, mean residence time of the disposition;Tl/2a, half-life of the absorption; Tl/2e(1), half-life elimination (phase 1); Tl/2e(2), half-life elimination (phase 2); Tlag a, lag time between administration and the onset of absorption.

[a] Experimental details for each group are shown in Table 1.

excretion via urine in bile-duct cannulated rats compared with intact rats suggested that a substantial proportion of the radiolabel excreted via the urine in intact rats must have been initially excreted via the bile and subsequently reabsorbed from the gastrointestinal tract.

The following inter-group comparisons were made. As discussed above, the rate and extent of excretion via urine was higher in females than in males receiving a single lower dose, and to a lesser extent also with a higher dose or a repeated lower dose. Males receiving a higher dose showed greater excretion via the faeces and less via the urine compared with males receiving the lower dose, which would be consistent with some saturation of absorption occurring (though substantial absorption was still indicated by the bile data, as discussed above). Similar results but with smaller differences were recorded in females at the higher dose. Comparing single with repeaetd lower doses, less excretion via the urine and more excretion via the faeces was indicated in rats receiving repeated doses, which would be consistent with slightly lower oral absorption after repeated doses or slightly modified metabolism. However, the differences involved were small in magnitude in males and even smaller in females (Justus, 2001a).

Metabolism

No major differences in metabolism were identified between the phenyl- and triazole-labelled prothioconazole. Only one metabolite was uniquely identified by the triazole label: 1,2,4-triazole (M13) at up to 2% of the administered dose in the urine. No triazole-free metabolites were identified in the experiements with phenyl-labelled prothioconazole.

Extensive metabolism was indicated, with a total of 18 metabolites (including the parent compound) identified in the urine, faeces or bile. The total proportion of the administered dose identified in each group ranged between 26% and 63%.

A further proportion of the administered dose (ranging from 9% to 43%) was characterized by high-performance liquid chromatography (HPLC). The largest component of the characterized material was a series of peaks grouped closely together in faeces samples that appeared to contain several metabolites that the study authors tentatively characterized as hydroxylated or conjugated faecal metabolites and called "faecal metabolite group 1".

The remainder of the material characterized by HPLC consisted of several minor faecal metabolites, the largest fraction of which consisted of < 5% of the administered dose in each of the groups without bile-duct cannulation. Faecal-extraction recovery rates were 67–79% of the administered dose, and attempts to improve on this rate were not successful.

Within the groups of bile-duct cannulated rats (groups 4 and 11), the largest peaks containing unknown material were three that contained multiple metabolites. Metabolites in one of the three peaks were identified as prothioconazole-desthio-hydroxy-glucuronide (M75), prothioconazole-desthio-dihydroxy-glucuronide (M72) and prothioconazole-desthio-hydroxy-methoxy-glucuronide (M49), but no metabolites could be identified in the other peaks.

All metabolites present in the total excreta at ≥ 5% of the administered dose were identified, excepting the unextractable faecal material and the metabolites in faecal metabolite group 1 (which were characterized). It appeared that all the main metabolites that could be extracted from excreta were identified.

Three standards for plant metabolites were available: prothioconazole-desthio-α-hydroxy (M18), prothioconazole-benzyl-propyldiol (M09) and prothioconazole-sulfonic acid (M02); these metabolites could not be identified unambiguously in the excreta of rats.

While the metabolites identified in the bile were mainly glucuronic acid conjugates, most of the material found in the faeces was not conjugated. Unchanged prothioconazole was the major metabolite found in the faeces, and was also found in small amounts in the urine and bile. The most abundant metabolite in bile of male rats and urine of male and female rats was the *S*-glucuronide of prothioconazole. Female rats excreted larger amounts of unchanged prothioconazole and its desthio metabolite via the faeces at the expense of memeebrs of faecal metabolite group 1.

The only difference in metabolism between a higher dose and a lower dose was greater excretion of unchanged prothioconazole and prothioconazole-desthio via the faeces in males at the higher dose. There was no notable difference between females receiving the higher or lower dose and the pattern of metabolites excreted by males receiving the higher dose was broadly similar to that of females receiving either the lower or higher dose.

Repeated dosing in males resulted in greater excretion of prothioconazole at the expense of memebers of faecal metabolite group 1, with no other notable differences. The reverse was true in females given repeated doses, though differences were small.

Overall, the most abundant metabolite was prothioconazole-*S*- or prothioconazole-*O*-glucuronide, which occurred at about 46% of the administered dose in the bile. The next most abundant metabolites were the parent compound, prothioconazole, which represented about 1–22% of the administered dose, and prothioconazole-desthio, which represented about 0.4–18% of the administered dose. Bile metabolites are ultimately excreted in the faeces. These amounts in the gastrointestinal tract are not available systemically as they undergo intensive enterohepatic circulation. This could also be shown in the autoradiograms obtained after administration of prothioconazole-desthio, a major plant residue of prothioconazole (Koester, 2001; Klein, 1991; described below).

The major types of metabolic reactions identified were conjugation with glucuronic acid, oxidative hydroxylation of the phenyl moiety and desulfuration. A proposed metabolic pathway is presented in Figure 2.

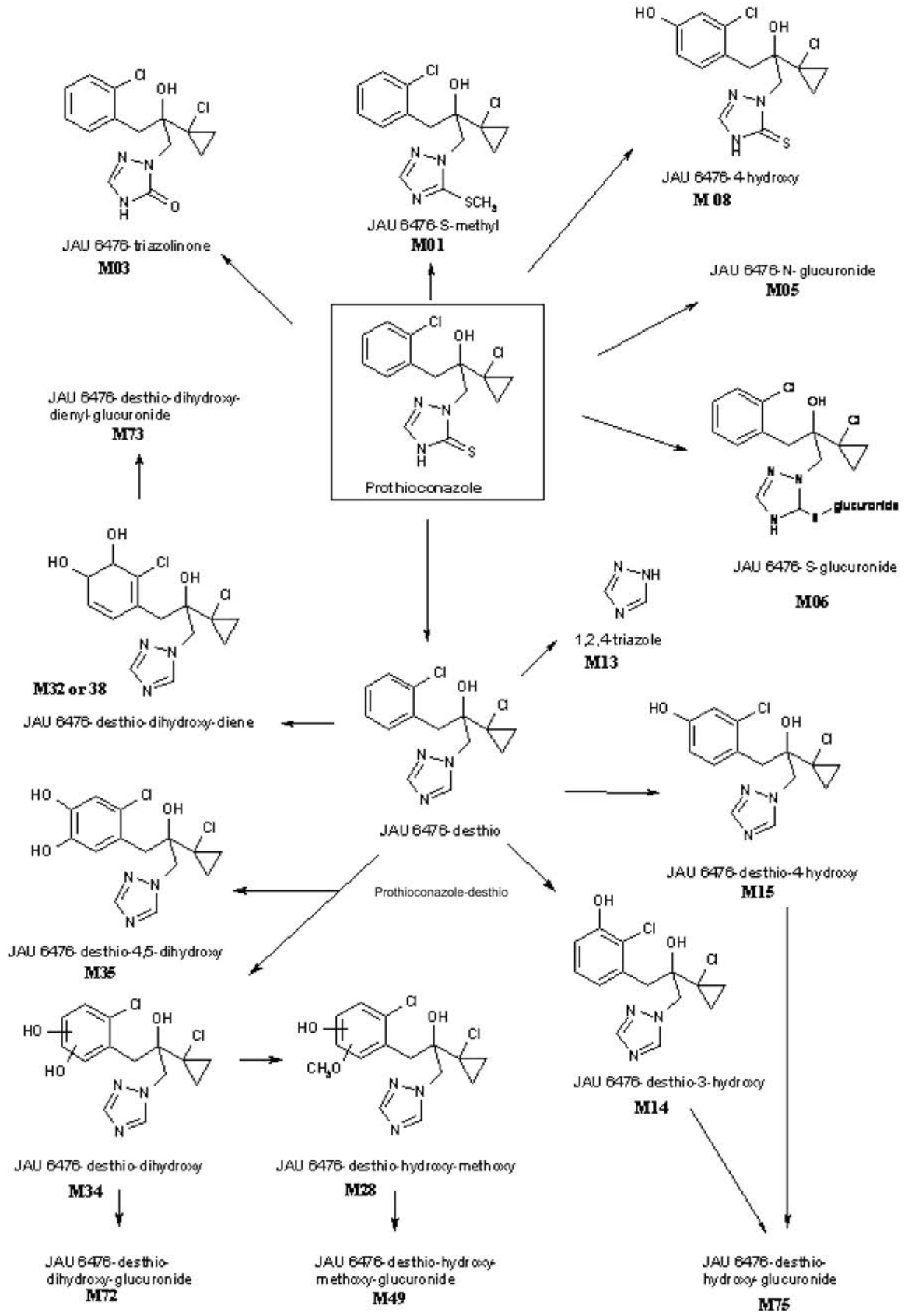


Figure 2. Metabolic pathways of prothioconazole

2. Toxicological studies

2.1 Acute toxicity

(a) Oral administration

The acute oral toxicity of prothioconazole (purity, 99.8%) was evaluated in groups of three male and three female Wistar rats given a single dose at 5000 mg/kg bw by gavage in 2% v/v aqueous Cremophor EL in a study that complied with OECD guideline 423 (1996). The test formulation was described as a very thick suspension. Stability and homogeneity were confirmed by analysis, but the test concentration was found to be 24% higher than nominal, therefore the true dose administered was 6200 mg/kg bw. The rats were observed for 14 days before autopsy.

There were no deaths. Clinical signs were limited to decreased motility and diarrhoea in all rats 1–6 h after dosing. Body-weight gains were normal and there were no gross findings at autopsy. The oral median lethal dose (LD_{50}) was > 6200 mg/kg bw (Andrews, 1998).

(b) Dermal administration

The acute percutaneous (dermal) toxicity of prothioconazole (purity, 98.8%) was investigated in groups of five male and five female Wistar rats given the test material at a dose of 2000 mg/kg bw as a powder moistened with water to the shaved skin under a semi-occlusive dressing for 24 h, according to OECD guideline 402 (1987). After the exposure period, the treated area was cleaned with soap and water. The rats were observed for 14 days and then killed and subjected to autopsy.

There were no deaths and no clinical signs of toxicity. Partial reddening of the treated skin (with partial scale formation in the case of females) was recorded over days 2–8. Body-weight gains were modest in males and absent in females. This could be attributed to the 24 h restraint period and the fact that females were aged 15 weeks (when significant body-weight gain would not be expected). There were no gross findings at autopsy. The dermal LD_{50} was > 2000 mg/kg bw (Kroetlinger, 1999).

(c) Inhalation

A test for the acute toxicity of prothioconazole (purity, 98.8%) administered by inhalation was conducted in a group of five male and five female Wistar rats that received a nose-only exposure to a solid aerosol of the test material at a concentration of 4.90 mg/l for 4 h. Prothioconazole was micronized before use, resulting in a decrease in purity to 92%. The micronized powder was aerosolized as a dust via a dust-feed generator without the use of a vehicle. The target concentration was 5.00 mg/l and the actual concentration (determined by gravimetric analysis) was the mean of 4.90 mg/l (4.79, 4.98, 5.13 and 5.06 mg/l at hourly intervals). The particle-size distribution in the atmosphere was determined gravimetrically twice during exposure using a nine-stage cascade impactor. The mass median aerodynamic diameter (MMAD) was 3.85 ± 2.06 μm geometric standard deviation (GSD). An additional untreated control group received conditioned air only. Detailed observations were made on the rats after the exposure period. These consisted of observations of rectal temperature, visual placing response, grip strength on wire mesh, abdominal muscle tone, corneal and pupillary reflexes, pinnal reflex, righting reflex, tail-pinch response and startle reflex stimulated by both sound and touch. The rats were observed for 14 days after exposure and before autopsy. The test atmosphere conditions were developed in preliminary trials to determine the 'maximum technically attainable' concentration while maintaining an aerosol particle-size distribution in the respirable range. The data showed that the exposure conditions were stable throughout the exposure period, and the particle-size distribution was acceptable.

There were no deaths. There were no clinical signs of toxicity in the control group. Piloerection, absence of grooming, bradypnoea, laboured breathing, serous nasal discharge, and reduced mobility occurred in all exposed rats on the day of exposure. Three males and three females also showed red encrustation around the muzzle or nostrils. All clinical signs had resolved within 3 days after exposure. There were no notable observations in the battery of reflex measurements made in the post-exposure period.

The mean rectal temperatures immediately after exposure (within 30 min of the end of the exposure period) were significantly lower in the test groups ($p < 0.01$ for males and females) than in the controls (Table 6). Slight, transient body-weight loss occurred during the first 3 days after exposure in males and females at 5.00 mg/l, compared with rats in the control group. Thereafter, the rats receiving prothioconazole gained weight at a comparable rate to rats in the control group. At necropsy, there were no treatment-related gross lesions in any of the rats receiving prothioconazole. The change in breathing rate and decreased body temperature were attributed to a non-specific response to sensory irritation from exposure to dust (the cited literature described a decline in metabolic rate and body temperature in rodents after inhalation of respiratory irritants).

In this study, there were no deaths and no marked toxicity induced by the maximum attainable concentration of the test material as a solid aerosol. The median lethal concentration (LC_{50}) was > 4.90 mg/l (Pauluhn, 1999). No classification for acute inhalation toxicity is required.

(d) Dermal irritation

Prothioconazole (purity, 99.8%) was evaluated for acute dermal irritation potential in three male Himalayan rabbits in a study conducted according to OECD guideline 404 (1992). Prothioconazole (500 mg) was moistened with water and applied to the test site (approximately 6 cm^2) and covered with a gauze pad that was held in place by a semi-occlusive dressing. The surrounding untreated skin served as the control. After 4 h, the pads were removed. No washing of the skin after exposure was described in the report. The rabbits were observed for skin reactions at 1, 24, 48 and 72 h after pad removal. Dermal irritation was scored and recorded according to the Draize method. There were no skin reactions in any rabbit at up to 3 days after exposure (Leuschner, 1999a).

(e) Ocular irritation

Prothioconazole (purity, 99.8%) was evaluated for acute eye irritation potential in three male Himalayan rabbits in a study conducted according to OECD guideline 405 (1987). Each rabbit received 100 mg of prothioconazole powder in the conjunctival sac of the right eye; the lower lid being gently pulled away from the eyeball to enable the substance to be administered. The lid was

Table 6. Body weights and rectal temperature in rats exposed to prothioconazole by inhalation for 4 h

Dose (mg/l air)	Group mean body weight (g)				Mean rectal temperature (°C)
	Day 1	Day 4	Day 8	Day 15	
Males					
0 (control)	199	211	233	263	37.9
4.90	194	187	208	236	34.0**
Females					
0 (control)	179	181	189	195	38.1
4.90	176	169	175	184	33.9**

From Pauluhn (1999)

** $p < 0.01$

then gently held together for about 1 s in order to prevent loss of test material. The left eye, which remained untreated, served as a control. No washing of the eye after exposure was described in the report. The eyes were examined at 1, 24, 48 and 72 h after dosing. At 24 h after dosing, the eyes were treated with fluorescein and examined. There were no corneal or iridial effects at any observation time. Minimal conjunctival redness (grade 1) was observed in one rabbit at 1 h, but not at any later time-point (Leuschner, 1999b).

(f) Dermal sensitization

The potential of prothioconazole (purity, 99.8%) to produce skin sensitization in female Hsd Poc:DH strain Hartley guinea-pigs was examined using the Magnusson and Kligman maximization test according to OECD guideline 406. Ten guinea-pigs received prothioconazole and five guinea-pigs were used for the control group. Prothioconazole was formulated as a suspension in sterile physiological saline solution containing 2% Cremophor EL. The stability and homogeneity of the test-material formulations were verified by analysis. The results of a contemporary test with the positive control (2-mercaptobenzothiazole) were acceptable.

Test concentrations were determined in a range-finding test. A single guinea-pig was treated twice with prothioconazole at a concentration of 5% by intradermal injection. A red weal developed after 24 h. A group of four guinea-pigs was exposed by occluded topical application for 24 h to prothioconazole at a concentration of 0%, 12%, 25% or 50% and dermal reactions were evaluated 24 h and 48 h after patch removal. A positive (grade 1) skin reaction occurred in one guinea-pig receiving prothioconazole at 25% and in three guinea-pigs at 50%. The maximum non-irritant concentration for topical challenge was determined in a group of five guinea-pigs previously induced topically and intradermally; the guinea-pigs in this group were treated with prothioconazole at a concentration of 0%, 12%, 25% or 50% by occluded topical application for 24 h. Dermal reactions were evaluated 24 h and 48 h after patch removal. A positive (grade 1) skin reaction occurred in three guinea-pigs at 25% and in three guinea-pigs at 50%. On the basis of these results, the following concentrations of prothioconazole for use in the main study were selected: intradermal induction, 5%; topical induction, 25%; topical challenge, 12%.

At the end of the topical induction and challenge phases, the prothioconazole remaining at the application site was removed with sterile physiological saline solution.

After the challenge application, minimal skin irritation (grade one on a four-point scale) was recorded in one guinea-pig at 24 and 48 h after exposure to prothioconazole. There was no irritation recorded in guinea-pigs in the control group. On the basis of these results (a positive response in 1 out of 10 guinea-pigs), prothioconazole was not considered to be a skin sensitizer (Stropp, 1999).

The potential of prothioconazole to produce skin sensitization was assessed in a local lymph node assay that complied with OECD guideline 406, OECD 429, 96/54/EC, Method B.6., B.42, and US EPA 712 -C-03-197, OPPTS 870.2600. Groups of six female Hsd Win:NMRI mice were treated with prothioconazole (purity, 97.2%) at a concentration of 0%, 2% 10% or 50% dissolved in dimethylformamide. On three consecutive days, epicutaneous applications of 25 µl per ear were made to the dorsal part of both ears. The composition of the test material corresponded to the specified limits of material from commercial large-scale production. The observed parameters included ear swelling, ear weight, local-lymph-node weight, cell-count determination, local-lymph-node-assay stimulation index and body weight (at the beginning and end of the study).

The mice did not show an increase in the stimulation indices for cell counts or for weights of the draining lymph nodes. The "positive level" of 1.4 for the cell-count index was never reached or exceeded in any group receiving prothioconazole. The "positive level" of ear swelling (an increase of 2×10^{-2} mm, i.e. about 10% of the control values) was not reached or exceeded in any group receiving prothioconazole. No increases in ear weights occurred when compared with mice in the control

group. Body weights were not affected. On the basis of these results, prothioconazole has no sensitizing potential (Vohr, 2007)

2.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female CD-1® mice were given prothioconazole (purity, 97.6%) at a dose of 0, 25, 100 or 400 mg/kg bw per day by gavage in 0.5% aqueous Tylose for 14 weeks. The homogeneity, stability and accuracy of the dosing solutions were confirmed by analysis. Haematology and blood chemistry investigations were performed at the end of the study. A gross autopsy was performed on all mice, and brain, liver, spleen, kidneys, adrenal glands and testes were weighed. After autopsy, phase I and II enzyme activities were measured in liver samples from five males and five females per group. Histopathology was performed for the liver of mice from all groups, all gross findings and selected organs from the control group and the group at the highest dose.

There were no treatment-related deaths and no notable clinical signs of toxicity. Body weights of treated males tended to be lower than those of mice in the control group, but differences were less than 10% and were compounded by a slightly higher initial mean body weight of males in the control group (2–4% higher immediately before treatment). Body weights of males at the highest dose were statistically significantly lower than those of mice in the control group in weeks 5, 10 and 11, but given the small magnitude and the pretreatment differences, the body-weight effects were not considered to be toxicologically significant. Body weights of females were similar to those of mice in the control group. There were no effects of treatment on food consumption.

There were no treatment-related, statistically significant changes in haematological profiles at any dose. At 400 mg/kg bw per day, erythrocyte and leukocyte cell counts, values for erythrocyte volume fraction and male haemoglobin concentration and lymphocyte/neutrophil ratios were slightly lower than control values, but remained within the normal range of background variation. Blood chemistry investigations did not reveal increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT) or alkaline phosphatase (ALP), but there was a 41%, statistically significant ($p < 0.01$ by adjusted Welch test) increase in plasma cholesterol concentration in females at the highest dose (control, 2.67 ± 0.49 µmol/l; 400 mg/kg bw per day, 3.77 ± 0.73 µmol/l**)**. At the highest dose there were also statistically significant decreases in concentration of bilirubin in males (42%) and females (28%) (males: control 1.9 ± 0.37 µmol/l; 400 mg/kg bw per day, 1.1 ± 0.25 µmol/l; females: control, 1.8 ± 0.25 µmol/l; 400 mg/kg bw per day, vs 1.3 ± 0.16 µmol/l) and in the concentration of total protein in males (control, 60.5 ± 0.90 g/l vs 57.6 ± 3.44g/l) ,which could be totally accounted for by decreased concentrations of albumin (control, 26.3 ± 1.82 g/l vs 23.4 ± 2.07 g/l). There was no effect of treatment on creatinine or urea. Electrolytes were not investigated.

At autopsy, enlargement of the liver was recorded in 3 out of 10 males and 1 out of 10 females at 400 mg/kg bw per day. Distinct liver lobulation was recorded in 1 out of 10 males treated at 100 mg/kg bw per day and in 5 out of 10 males treated at 400 mg/kg bw per day. There were no other notable autopsy findings. The only significant organ-weight effects were in the liver, with increases in absolute and relative liver weights in all treated males and in females at 100 and 400 mg/kg bw per day. The absolute liver-weight increases in the groups at 25, 100 and 400 mg/kg bw per day, respectively, were 108%, 113% and 144% in males and 102%, 114% and 139% in females.

The only treatment-related histopathological findings were in the liver (Table 7).

Hepatocellular hypertrophy with cytoplasmic change was recorded in males and females at 100 and 400 mg/kg bw per day with severity increasing with dose. Liver-cell enlargement was characterized by karyomegaly and cytomegaly predominantly located in the centre of the liver lobule. The cytoplasmic change was described as ‘a fine granular and/or foamy structure’. Cytoplasmic

vacuolation (with vacuoles varying in size from a micro- to a macrovesicular, commonly due to an accumulation of triglycerides) was recorded in males at 400 mg/kg bw per day. Oil red O staining revealed a dose-related increase in the severity of hepatocellular centrilobular fatty change in males at 100 and 400 mg/kg bw per day and of periportal fatty change in females at 400 mg/kg bw per day. All other histopathological findings were considered to be incidental.

The activities of all microsomal enzymes measured were increased in a dose-related pattern in all treated groups of females (Table 8). Increases were also found in most enzymes in treated males at 100 and 400 mg/kg bw per day, but the increases were less marked.

The liver was clearly identified as the target organ for prothioconazole in mice. The liver histopathology was consistent with enzyme induction and was accompanied by increased liver weights and increases in microsomal liver-enzyme activities. Small increases in liver weights and liver enzyme activities at 25 mg/kg bw per day were not considered to be adverse owing to the small magnitude of the changes and the absence of accompanying histopathological findings.

The NOAEL was 25 mg/kg bw per day on the basis of histopathological findings in the liver at 100 mg/kg bw per day (Wirnitzer & Hartmann, 1999a).

Rats

Groups of five male and five female Wistar rats were given diets containing prothioconazole technical (purity, 99.5%) at nominal concentrations of 0, 400, 2000 or 10 000 ppm, equal to 0, 18.6, 146 and 952 mg/kg bw per day for males and 0, 18.8, 151 and 1033 mg/kg bw per day for females, for 4 weeks. Diets were frozen after preparation and were changed every 3–4 days. The stability of prothioconazole in the diet and the homogeneity of the dietary mixtures were verified before the start of the study. Analyses for correct concentration and homogeneity were satisfactory at 10 000 ppm,

Table 7. Histopathological findings in the liver of mice given prothioconazole by gavage for 14 weeks

Finding	No. of mice affected (mean severity[a])							
	Dose (mg/kg bw per day)							
	Males				Females			
	0	25	100	400	0	25	100	400
Gross lesions								
No. of livers examined	10	10	10	10	10	10	10	10
Overt liver lobulation	0	0	1	6[b]	0	0	0	0
Enlarged liver	0	0	0	3	0	0	0	1
Cytoplasmic change	0	0	9 (1.6)	9 (2.3)	0	0	3 (1.0)	10 (1.4)
Hypertrophy	0	1 (1.0)	9 (1.9)	9 (2.7)	0	0	3 (1.0)	10 (1.4)
Vacuolation	1 (1.0)	0	1 (3.0	6 (2.0)	0	0	0	1 (2.0)
Focal necrosis	0	0	0	3	1	0	0	2
Fatty change:								
Centrilobular	8 (1.0)	5 (1.0)	10 (1.6)	10 (2.6)	0	3 (1.0)	5 (1.0)	2 (1.5)
Periportal	0	0	0	0	1 (1.0)	1 (1.0)	0	6 (1.5)
Diffuse	0	0	0	0	4 (1.3)	3 (1.7)	3 (1.3)	0

From Wirnitzer & Hartmann (1999a)

[a] Severity of findings is graded on a five-point scale (1 = minimal, 5 = extensive).

[b] Includes one male that died during the blood-sampling procedure.

but neither the achieved concentrations nor stability were acceptable at 400 and 2000 ppm. The calculated actual concentrations of prothioconazole in the diet are shown in Table 9.

Since prothioconazole was unstable in the diet, a linear degradation rate with time was assumed and the stability data used, with measured food consumption, to calculate the actual doses received. Food consumption and body weight were determined once per week. The state of health of the rats was checked daily. Blood samples were taken from all rats for haematology and blood-chemistry examinations at the end of the study. The blood-chemistry investigations included the thyroid hormones triiodothyronine (T3), and thyroxin (T4). Urine was also analysed at the end of the study. All rats were subjected to complete gross examinations, and weights of selected organs were determined. Histopathology was performed on all tissues from the control group and the group at the highest dose and on liver, heart, lungs, kidneys and gross lesions from all groups. Additional samples of liver and kidney were shock-frozen at −140 °C for determination of the cell proliferation index according to proliferating cell nuclear antigen (PCNA) measurements. Further liver samples were retained from all rats for the determination of hepatic triglycerides and phase I and II enzymes.

There were no deaths and no clinical signs of toxicity. Body-weight gains were markedly reduced in males at 10 000 ppm relative to those of rats in the control group (up to 40% lower each week), such that body weights at termination were 22% lower than those of those of rats in the control group. No effect was recorded on body weights in females. Food consumption was slightly higher than controls in males and females at 10 000 ppm, and water consumption was markedly increased

Table 8. Mean enzyme activities in liver of mice given prothioconazole by gavage for 14 weeks

Enzyme	Dose (mg/kg bw per day)							
	Males				Females			
	0	25	100	400	0	25	100	400
ECOD (nmol/g per min)	9.5	9.2	33.0*	36.5	15.8	20.9*	32.8**	48.7**
EROD (nmol/g per min)	0.48	0.54	1.21*	1.24	0.50	0.84*	1.76**	2.14**
ALD (nmol/g per min)	23.5	28.1	56.6*	54.0	21.7	31.6*	45.7**	98.7**
EH (nmol/g per min)	611	656	655	551	281	286	372	419**
GST (µmol/g per min)	591	482	574	620	117	137*	172**	257**
GLU-T (nmol/g per min)	241	153*	373	349	244	296	338	397*

From Wirnitzer & Hartmann (1999a)

ECOD, 7-ethoxycoumarin-deethylase; EROD, 7-ethoxyresorufin-deethylase; ALD, aldrin epoxidase; EH, epoxide hydrolase; GST, glutathione *S*-transferase; GLU-T, UDP-glucuronyltransferase.

$p < 0.05$; ** $p < 0.01$ (t-test).

Table 9. Calculated dietary concentrations of prothioconazole in a study in rats fed diets containing prothioconazole for 4 weeks

Nominal dietary concentration (ppm)	Percentage of nominal concentration found by analysis			Mean actual dietary concentration (ppm)
	Day 0	Day 3	Day 4	
400	68	47	32	196
2 000	84	—	64	1480
10 000	97	90	88	9250

From Andrews & Romeike (1997)

in males and females at 10 000 ppm (36% and 47% higher in males and females respectively). There were no notable haematological effects.

Blood-chemistry investigations revealed increases in a number of parameters in males and females, mainly at 10 000 ppm. While there was no significant change in AST activity, there were significant increases in ALT in males and females at 10 000 ppm (males: control, 42.2 ± 7.8; 10 000 ppm, 62.0 ± 9.3 U/l; females: control, 49.2 ± 8.2; 10 000 ppm, 68.2 ± 9.7 U/l) and ALP in males and females at 2000 and 10 000 ppm (males: control, 566 ± 52 vs 715 ± 64 U/l and 740 ± 96; females, 361 ± 32 vs 470 ± 63 and 489 ± 104 U/l). In addition, in the group receiving 10 000 ppm there were significant increases in cholesterol concentrations in males and females (males: control, 2.15 mmol/l; 10 000 ppm, 2.71 mmol/l; females: control, 2.10 mmol/l; 10 000 ppm, 2.86 mmol/l) and in urea concentrations (males: control, 7.05 vs 8.60 mmol/l; females, 7.34 vs 9.31 mmol/l). Females (but not males) at 10 000 ppm showed a statistically significant decrease in T4 concentrations (females: control, 49 nmol/l; 10 000 ppm, 24 nmol/l) and a statistically significant increase in thyroid-stimulating hormone (TSH) (females: control, 2.30 ng/ml; 10 000 ppm, 4.96 ng/ml). Changes in the concentration of these hormones did not reach significance in males, the measured values tending towards similar outcome at 10 000 ppm (T4, males: control, 54 nmol/l; 10 000 ppm, 42 nmol/l; TSH, males: control, 4.80; 10 000 ppm, 6.40 mg/ml. There was also a slight increase in plasma concentrations of calcium in females at 10 000 ppm. Urinary volume was slightly lower and urinary density slightly higher in males and females at 10 000 ppm.

Treatment-related gross lesions at autopsy included pale, discoloured and marbled kidneys in two males at 10 000 ppm. The absolute liver weights were significantly different from those of rats in the control group only in the group at 10 000 ppm. In males there was a decrease to 83% of the control value, while in females there was an increase to 120%. Other organ-weight changes were considered to be related to the reduced body weights of males at the highest dose.

Histopathology revealed treatment-related lesions in the kidneys of rats of males and females at 10 000 ppm (Table 10). The effect was characterized by an increase in the incidence and severity of basophilic tubules and cortical tubular dilatation and was more pronounced in male rats. The effect was not observed at lower doses. All other microscopic findings were considered to be incidental to treatment with prothioconazole. There were no lesions of the thyroid gland to account for the changes in thyroid hormone levels; this indicates a physiological compensatory mechanism in the thyroid rather than a toxic effect. There were also no histopathological findings in the liver to correlate with the changes in liver weight and the clinical-chemistry findings.

Table 10. Incidence and severity of treatment-related histopathological alterations in the kidneys of rats fed diets containing prothioconazole for 4 weeks

Lesion	Dietary concentration (ppm)							
	Males				Females			
	0	400	2000	10 000	0	400	2000	10 000
Basophilic tubules:								
No. examined	5	5	5	5	5	5	5	5
Incidence	1	0	0	5	0	0	0	3
Mean severity[a]	1.0	—	—	3.8	—	—	—	2.3
Tubular dilatation:								
No. examined	5	5	5	5	5	5	5	5
Incidence	0	0	0	5	0	0	0	2
Mean severity[a]	—	—	—	2.0	—	—	—	2.0

From Andrews & Romeike (1997)

[a] Severity of findings is graded on a five-point scale (1 = minimal, 5 = extensive).

The cell-proliferation index was estimated in liver and kidney samples by measuring the frequency of proliferating cell nuclear antigen (PCNA)-containing cells (Table 11). Separate analyses were performed for periportal and perivenular areas in the liver and for cortex and medulla in the kidney. Analysis of liver of males and females and the kidney of females was restricted to the control group and the group at 10 000 ppm, while the kidneys of males in the group at 2000 ppm were also analysed. Cell proliferation was reduced by approximately 20–40% compared with values for controls in both areas of the liver of males and females at 10 000 ppm. In the kidney, there were marked increases in males at 10 000 ppm in both cortex and medulla (+ 306% and + 229%, respectively), but in males at 2000 ppm there were slight reductions (22% and 31% lower than values for controls). Females at 10 000 ppm showed a higher proliferation index in the medulla, but no effect in the cortex, the increase in the medulla being 88% if the exceptionally high value (+ 700%) from one out of the five rats is excluded. Other than describing this exception, no data for individual rats were provided. The study authors concluded that these results indicated a toxic effect rather than a primary proliferative effect; however, there was no mention of cell death in the description of the histopathology. The results from this investigation correlated with the observation of basophilia in the kidneys of rats at 10 000 ppm.

Additional liver samples were analysed for a number of enzyme activities (Table 12). There was weak induction of cytochrome P450 activity in males and females at 10 000 ppm, but the only oxygenase activity that was increased was that of 7-ethoxycoumarin deethylase (ECOD) activity in males (and not in females). Significantly decreased activities were observed in the activities of ethoxyresorufin deethylase (EROD) in males and females at 10 000 ppm. In male rats there were significant decreases in aldrin epoxidase (ALD) activity at all doses and significant increases in epoxide hydrolase (EH) activity, again at all doses. Dose-related effects on these enzyme activities in female rats were restricted to the rats at 10 000 ppm. Glutathione *S*-transferase (GST) and UDP-glucuronyltransferase (GLU-T) were increased in males and females at 2000 and 10 000 ppm.

This study demonstrated treatment-related effects at 10 000 ppm. These were reductions in body-weight gain, increases in food and water consumption, increases in liver weights, increases in plasma cholesterol (in females) and urea and in ALT and ALP activities, changes in plasma thyroid-hormone concentrations (in females) and changes in renal histology. There were also changes in the activities of hepatic enzymes involved in both phase I and phase II metabolism. Some similar observations were made in rats at 2000 ppm. These included an increase in plasma ALP activity and changes in the activities of hepatic enzymes involved in both phase I and phase II metabolism. The latter were also affected in rats at 400 ppm. Since these changes in hepatic enzyme activities were not accompanied by histological changes at either 400 or 2000 ppm, they were considered to be

Table 11. Group mean cell proliferation index[a] (% of control value) in rats fed diets containing prothioconazole for 4 weeks

Sex	Dietary concentration (ppm)	Group mean cell proliferation index (% of value for controls)			
		Liver		Kidney	
		Periportal	Perivenous	Cortex	Medulla
Male	10 000	–22	–24	+306	+229
	2 000	NE	NE	–22	–31
Female	10 000	–42	–28	–9	+88[b]

From Andrews & Romeike (1997)

NE, not evaluated.

[a] No. of PCNA-positive cells per 1000 cells.

[b] Excludes the result for one unrepresentative rat (proliferation index, + 700%).

Table 12. Group mean enzyme activities in rat liver after 4 weeks dietary treatment with prothioconazole

Enzyme	Dietary concentration (ppm)							
	Males				Females			
	0	400	2000	10 000	0	400	2000	10 000
P450 (nmol/g) ± SD	34.0 ± 3.8	32.5 ± 3.2	37.7 ± 5.2	49.6** ± 7.4	31.1 ± 2.8	31.0 ± 3.4	32.4 ± 5.1	43.3** ± 7.0
ECOD (nmol/g per min) ± SD	6.1 ± 1.4	6.6 ± 1.2	6.1 ± 1.7	9.3** ± 1.6	2.7 ± 0.6	2.0 ± 0.5	2.1 ± 0.7	3.0 ± 0.6
EROD (nmol/g per min) ± SD	0.65 ± 0.33	0.52 ± 0.30	0.49 ± 0.21	0.23* ± 0.14	0.34 ± 0.16	0.16 ± 0.07	0.28 ± 0.12	0.15* ± 0.08
ALD (nmol/g per min) ± SD	146.6 ± 22.5	85.7** ± 16.6	82.1** ± 28.2	52.2** ± 8.9	25.2 ± 5.0	16.5* ± 3.6	18.4 ± 5.6	16.4* ± 4.8
EH (nmol/g per min) ± SD	338 ± 29	441** ± 45	718* ± 254	856** ± 205	293 ± 43	360 ± 108	379 ± 99	666** ± 127
GST (µmol/g per min) ± SD	67 ± 5	70 ± 8	90* ± 19	103** ± 11	57 ± 5	59 ± 3	71** ± 5	102** ± 13
GLU-T (nmol/g per min) ± SD	452 ± 97	528 ± 78	621 ± 187	612* ± 63	193 ± 30	195 ± 52	259* ± 53	411** ± 128

From Andrews & Romeike (1997)

ECOD, 7-ethoxycoumarin-deethylase; EROD, 7-ethoxyresorufin-deethylase; ALD, aldrin epoxidase; EH, epoxide hydrolase; GST, glutathione *S*-transferase; GLU-T, UDP-glucuronyltransferase; SD, standard deviation.

$p < 0.05$; ** $p < 0.01$ (t-test); ± standard deviation.

adaptations to the presence of prothioconazole in the diet. Also, there were no histological changes accompanying the changes in plasma enzyme acitivities at 2000 ppm or, more specifically, any changes in thyroid histology accompanying the changes in plasma thyroid-hormone concentrations.

The NOAEL was nominally 2000 ppm, or 1480 ppm after taking test-material degradation into account, equal to 146 mg/kg bw per day, on the basis of reduced body-weight gain, increased liver weights, more marked clinical-chemistry findings and histopathological findings in the kidneys at, nominally, 10 000 ppm, or 9250 ppm after taking degradation into account, equal to 952 mg/kg bw per day (Andrews & Romeike, 1997).

Groups of five male and five female Wistar rats were given prothioconazole technical (purity, 99.5%) in a 4-week study of the effect of different modes of dose administration on the toxicity of the compound. Two groups received diets containing prothioconazole at a concentration of 0 or 10 000 ppm, equal to doses of 1 038–1 067 mg/kg bw per day. As an important part of this study, a third group received diet containing silica-stabilized prothioconazole (8.25% prothioconazole in silica gel at a concentration of 10 000 ppm, equal to doses of 850–1 078 mg/kg bw per day). Two additional groups received prothioconazole at a dose of 0 (vehicle only) or 1 000 mg/kg bw per day by gavage in 0.5% aqueous carboxymethylcellulose.

Diets were mixed twice per week and contained 1% peanut oil to minimize dust formation. The prothioconazole content of the test materials was confirmed by analysis (for single samples on the day of preparation).

This study included a restricted range of investigations on the rats receiving prothioconazole in the diet or by gavage that could be used as a link to the more complete 4-week dietary study described above (Andrews & Romeike, 1997). However, a wider range of investigations (including haematology, clinical chemistry, organ weights and histopathology on a full range of tissues) was carried out on the rats dosed by gavage. In addition, plasma samples taken after 1, 7, 14 and 21 days of treatment were analysed for concentrations of prothioconazole and the metabolite prothioconazole-desthio to determine the effect of mode of dosing on kinetics.

The plasma concentrations of prothioconazole and prothioconazole-desthio were measured after 1, 7, 14 and 21 days of treatment (Table 13). Blood from rats treated by gavage was sampled approximately 2 h after dosing, rats receiving diet containing prothioconazole were sampled at around 08:00.

Steady-state plasma concentrations were attained in males and females after 14 days of treatment. Steady-state plasma concentrations were lowest with silica-stabilized dietary prothioconazole (11.0–19.6 nmol/ml), slightly higher with dietary non-stabilized prothioconazole (25.0–49.6 nmol/ml) and were markedly higher in the rats treated by gavage (66.3–108.3 nmol/ml). Non-stabilized dietary prothioconazole resulted in plasma concentrations that were approximately twofold those obtained with silica-stabilized dietary prothioconazole, while treatment by gavage were approximately sixfold. The rats dosed by gavage showed a high initial concentration, which decreased by approximately half at 7 days, and decreased slightly further to steady state by 14 days. Concentrations in females were consistently higher than concentrations in males. The results for the metabolite prothioconazole-desthio showed the same pattern as for prothioconazole, but the concentrations were lower (approximately 2–3% of the concentrations determined for prothioconazole).

There were no deaths. The only clinical sign of toxicity was piloerection in males and females treated with non-stabilized prothioconazole in the diet or by gavage mostly during the first 2 weeks of the study. Food consumption was slightly higher in rats treated with non-stabilized prothioconazole. There was no similar effect in rats reated with silica-stabilized prothioconazole. Water consumption was markedly higher in rats receiving non-stabilized prothioconazole in diet, but not silica-stabilized material. Water consumption was not measured in rats dosed by gavage. Reduced body-weight gains

Table 13. Group mean plasma concentrations of prothioconazole in rats treated with prothioconazole (silica-stabilized or non-stabilized) in the diet or by gavage for 4 weeks

Test material	Route	Dose/dietary concentration	Mean plasma concentration of prothioconazole (nmol/ml) ± standard deviation[a]			
			1 day	7 days	14 days	21 days
Males						
Silica-stabilized	Diet	10 000 ppm	29.1 ± 6.9	14.8 ± 3.0	13.2 ± 5.3	11.0[a] ± 3.3
Non-stabilized	Diet	10 000 ppm	30.6 ± 6.4	38.4 ± 14.6	33.4 ± 10.5	25.0 ± 2.6
Non-stabilized	Gavage	1 000 mg/kg bw per day	178.4 ± 29.6	97.3 ± 23.9	67.7 ± 11.7	66.3 ± 27.3
Females						
Silica-stabilized	Diet	10 000 ppm	34.7 ± 13.6	21.8[a] ± 7.2	19.6 ± 11.2	18.6 ± 9.8
Non-stabilized	Diet	10 000 ppm	46.0 ± 16.1	42.3[a] ± 11.7	49.6 ± 11.1	48.5[a] ± 6.1
Non-stabilized	Gavage	1 000 mg/kg bw per day	224.6 ± 26.2	119.4 ± 16.9	97.8 ± 27.6	108.3 ± 18.1

From Andrews et al. (1998)

[a] No prothioconazole was detected in the plasma of rats receiving control diet or vehicle only.

[b] Excludes one aberrant value.

were recorded in treated rats, particularly in males. Male rats dosed by gavage or fed non-stabilized prothioconazole were affected more than males and females fed silica-stabilized material. There was no discernible effect of silica on the reduction of body-weight gain seen in females receiving dietary prothioconazole.

Haematology and clinical chemistry investigations were restricted to rats dosed by gavage. Leukocyte counts were slightly higher in males treated in this way with prothioconazole, but they were within the range of historical control values and, overall, the results of haematology were unremarkable. Blood chemistry revealed slightly increased ALT activity in males and females, ALP activity in females and urea concentrations in males. These results are, in general, consistent with those in diet-fed rats in the previous 4 week study (Andrews& Romeike, 1997), except that males and females were consistently affected in that study.

Treatment-related changes in hepatic enzyme activities were observed in all rats receiving prothioconazole (Table 14). Qualitatively, the changes were very consistent with those of the previous 4-week dietary study (Andrews & Romeike 1997), i.e. increases in the activities of ECOD, EH,

Table 14. Group mean liver tissue enzyme activities in rats treated with prothioconazole (silica-stabilized or non-stabilized) in the diet or by gavage for 4 weeks

Test material	Route	Dose/dietary concentration	Enzyme activity ± standard deviation					
			ECOD (nmol/g per min)	EROD (nmol/g per min)	ALD (nmol/g per min)	EH (nmol/g per min)	GST (µmol/g per min)	GLU-T (nmol/g per min)
Males								
Control diet	Diet	0	4.8 ± 0.7	0.37 ± 0.12	84.6 ± 20.9	474 ± 65	103 ± 7	727 ± 135
Silica-stabilized	Diet	10 000 ppm	6.3* ± 1.2	0.41 ± 0.09	58.7 ± 15.5	804** ± 151	124* ± 13	1007* ± 154
Non-stabilized	Diet	10 000 ppm	7.7** ± 1.6	0.24 ± 0.13	38.9** ± 8.0	1090** ± 351	160** ± 17	974 ± 198
Vehicle[a]	Gavage	0	5.4 ± 1.2	0.38 ± 0.13	123.0 ± 8.2	391 ± 108	87 ± 4	801 ± 102
Non-stabilized[a]	Gavage	1 000 mg/kg bw per day	10.4** ± 2.7	0.39 ± 0.14	48.0** ± 9.6	1086** ± 302	139** ± 19	1070* ± 171
Females								
Control diet	Diet	0	2.0 ± 0.7	0.33 ± 0.19	16.9 ± 4.9	197 ± 34	89 ± 5	464 ± 159
Silica-stabilized	Diet	10 000 ppm	2.1 ± 0.4	0.27 ± 0.08	12.8 ± 3.3	281* ± 72	89 ± 11	614 ± 180
Non-stabilized	Diet	10 000 ppm	2.4 ± 0.9	0.19 ± 0.12	14.4 ± 5.7	570** ± 245	125** ± 15	644 ± 186
Vehicle[a]	Gavage	0	2.0 ± 0.4	0.24 ± 0.07	15.0 ± 3.2	224 ± 33	66 ± 11	393 ± 91
Non-stabilized[a]	Gavage	1 000 mg/kg bw per day	2.6 ± 0.7	0.17 ± 0.10	14.4 ± 2.7	466** ± 91	106** ± 7	625* ± 146

From Andrews et al. (1998)

ECOD, 7-ethoxycoumarin-deethylase; EROD, 7-ethoxyresorufin-deethylase; ALD, aldrin epoxidase; EH, epoxide hydrolase; GST, glutathione *S*-transferase; GLU-T, UDP-glucuronyltransferase.

[a] Vehicle was 0.5% aqueous carboxymethylcellulose. Vehicle control and dose were each delivered in a volume of 10 ml/kg bw.

* $p < 0.05$; ** $p < 0.01$ (t-test).

GST and GLU-T, but a decrease in the activity of ALD. The largest effects were induced by treatment by gavage, followed by treatment with non-stabilized prothioconazole, then treatment with silica-stabilized prothioconazole (although the differences between results for the two dietary forms were small).

Treatment-related histopathological effects were observed in the kidneys of male and female rats given non-stabilized prothioconazole in the diet and by gavage, but not in rats treated with silica-stabilized prothioconazole (Table 15). The effect was described as an increased frequency and/or severity of predominantly bilaterally occurring basophilic tubules, similar to the observations made in the earlier 4-week study (Andrews and Romeike, 1997). Minimal to slight cytoplasmic changes in centrilobular hepatocytes were also recorded at a higher frequency in rats receiving non-stabilized prothioconazole in the diet and by gavage.

The results confirmed that the liver and kidneys are the target organs for toxicity attributable to prothioconazole and clearly showed that the form in which prothioconazole is administered markedly influences the toxic effects. For dietary administration, stabilization of prothioconazole with silica lowers the steady-state plasma concentration compared with non-stabilized prothioconazole. Administration by gavage results in higher steady-state plasma concentrations compared with dietary administration. This in turn leads to rats treated by gavage showing the most frequent and severe effects and silica-stabilized prothioconazole producing the fewest and least severe effects.

The objectives and design of this study did not allow for estimation of an NOAEL (Andrews et al., 1998).

Table 15. Incidence of treatment-related histopathological alterations in rats treated with prothioconazole (silica-stabilized or non-stabilized) in the diet or by gavage for 4 weeks

Test material	Route	Dietary concentration or dose	Cytoplasmic change in the liver		Increased frequency and/or severity of basophilic tubules in kidneys	
			No. examined	No. affected	No. examined	No. affected (severity)[b]
Males						
Control diet	Diet	0 ppm	5	0	5	2 (1.0)
Silica-stabilized	Diet	10 000 ppm	5	1	5	1 (1.0)
Non-stabilized	Diet	10 000 ppm	5	0	5	5 (1.4)
Vehicle[a]	Gavage	0 mg/kg per day	5	0	5	2 (1.0)
Non-stabilized	Gavage	1 000 mg/kg per day	5	3	5	5 (1.8)
Females						
Control diet	Diet	0 ppm	5	0	5	0 (—)
Silica-stabilized	Diet	10 000 ppm	5	0	5	1 (1.0)
Non-stabilized	Diet	10 000 ppm	5	3	5	2 (1.5)
Vehicle[a]	Gavage	0 mg/kg per day	5	0	5	0 (—)
Non-stabilized	Gavage	1 000 mg/kg per day	5	3	5	5 (1.4)

From Andrews et al. (1998)

[a] Vehicle was 0.5% aqueous carboxymethylcellulose. Vehicle control and dose were each delivered in a volume of 10 ml/kg bw.

[b] Severity of findings is graded on a five-point scale (1 = minimal, 5 = extensive).

Groups of 10 male and 10 female Wistar rats were given prothioconazole (purity, 97.6%) at a dose of 0, 20, 100 or 500 mg/kg bw per day by gavage in 0.5% aqueous Tylose for 14 weeks. Additional animals (rats in the control group and rats at the highest dose) were maintained for a 4-week recovery period and a further satellite group of five male and five female rats were treated for 4 weeks prior to immunotoxicological investigations. The homogeneity, stability and accuracy of the dosing solutions were confirmed by analysis. Haematology and blood chemistry investigations were performed after 5 and 14 weeks (and also after 19 weeks for rats in the recovery group). Concentrations of thyroid hormones (T3, T4 and TSH) were determined. Plasma concentrations of prothioconazole and the metabolite prothioconazole-desthio were determined 1 h after dosing during weeks 6 and 11. A gross autopsy was performed on all rats. This included the weighing of a broad range of organs (except thymus and uterus), after which histopathology was performed on all tissues from the control group and the group at the highest dose, plus the liver, lungs, kidneys and gross lesions from all groups at the end of the study. After autopsy, phase I and II enzyme activities were measured in liver samples from five males and five females per group. At autopsy, half a spleen and a sample of blood were collected from six males and six females per group (from the main group) and the following parameters determined: cell count, subpopulation composition of lymphocytes (determined by fluorescence-activated cell sorting – FACS – analysis) and macrophage activity from spleen and IgA, IgG and IgM titres from the blood. The satellite groups of five males and five females were immunized intravenously with sheep erythrocytes 4 days before they were killed after 4 weeks of treatment in order to carry out plaque-forming cell assays (PFCA).

The plasma concentrations of prothioconazole and prothioconazole-desthio were measured 1 h after dosing on days during weeks 6 and 11 (Table 16). These data show that plasma concentrations in females are significantly higher(approximately twofold) in females than in males. The increases in plasma concentrations were proportional to dose between 20 and 100 mg/kg bw per day but were not completely proportional to dose between 100 and 500 mg/kg bw per day, where increases were approximately twofold over this fivefold increase in dose. Concentrations of prothioconazole-desthio were very low (approximately 0-4% of concentrations of prothioconazole).

Table 16. Group mean plasma concentrations of prothioconazole and prothioconazole-desthio in a short-term study in rats treated with prothioconazole by gavage

Dose (mg/kg bw per day)	Sex	Group mean plasma concentration (nmol/ml)	
		Prothioconazole	Prothioconazole-desthio
Week 6			
20	Male	2.7	0.1
100	Male	22.5	0.9
500	Male	28.1	1.6
20	Female	7.0	0.0
100	Female	31.9	0.5
500	Female	68.4	1.6
Week 11			
20	Male	2.1	0.2
100	Male	18.6	1.3
500	Male	41.4	1.4
20	Female	7.0	0.3
100	Female	38.3	0.8
500	Female	86.1	2.1

From Wirnitzer & Hartmann (1999a)

There was one death, a female at the highest dose, which did not appear to be related to misdosing or blood-sampling errors. This rat was killed while in a moribund condition on day 96. The cause of the condition was not clearly identified. Findings at autopsy were a dilated urinary bladder and histopathology findings were inflammation of the tongue and basophilic tubules in the kidney. Otherwise, there were no clinical signs and no significant ophthalmoscopic findings in rats at the highest dose at the end of the study.

Body weights of all rats receiving prothioconazole were similar to those of the controls throughout the study. Food consumption was not affected by treatment, but water consumption was increased by 20–24% in males and females at the highest dose compared with values for controls. During the 4-week recovery period, water consumption was similar to that of controls in males or only slightly higher than controls in females.

Haematology revealed no treatment-related effects. Blood chemistry investigations found that ALT and especially AST activities were slightly reduced at the highest dose in comparison to values for controls. This result contrasts with those of previous dietary studies in which the activities of these enzymes in plasma were increased. There was no effect on ALP activity. Plasma cholesterol concentrations were significantly increased in males and females at the highest dose in week 5 and in week 14. Triglyceride concentrations were consistently lower in females at the highest dose. Urea concentrations were significantly higher in males at the highest dose at week 14, but not at week 5. There were no significant blood-chemistry findings after the 4-week recovery period.

Thyroid hormones (T3, T4 and TSH) were analysed at week 5, week 14 and in rats in the recovery group. Rats in the group at the highest dose tended to have lower T4 concentrations than did the controls (sometimes accompanied by very slightly higher T3 concentrations), but the differences were small and either within or only slightly outside the normal range of reference values provided by the performing laboratory. No deviations in plasma TSH concentrations were recorded.

Urinary volume was slightly reduced in males at 500 mg/kg bw per day in weeks 4 and 13 and in females of the same group in week 4. There were corresponding small increases in urine density and protein concentration. There was no effect at 100 mg/kg bw per day and below, and no significant differences were apparent in any group after 4 weeks recovery.

The spleen-cell and peripheral-blood-lymphocyte (immunological) investigations did not yield any toxicologically significant results. There were no effects on the number and size distribution of spleen cells or on the subpopulation analysis of T-cells. The number of splenic B-cells and antigen presenting cells were significantly increased in females at 500 mg/kg bw per day. Splenic macrophages of males at 100 mg/kg bw per day and males and females at 500 mg/kg bw per day had a marginally higher activation status after stimulation with phorbol 12-myristate 13-acetate (PMA); however, specific-antibody induction and response (the PFCA assay) and peripheral blood IgA, IgM and IgG antibody titres were unaffected by treatment at 500 mg/kg bw per day. The effects that were seen were considered to be minor findings and, in the absence of any correlating histopathological or other findings and with no effect on the functional assay (the PFCA assay), the Meeting concluded that there was no effect of treatment with prothioconazole on the immune system.

Activities of microsomal liver enzymes were measured at the autopsies of rats of the main group (14 weeks) and the recovery group (18 weeks). In rats in the main group, EH was significantly increased at 500 mg/kg bw per day in males (120%) and females (85%), GLU-T was significantly increased at 500 mg/kg bw per day in males (51%), and ALD was significantly reduced in all male rats receiving prothioconazole. There were no consistent effects on ECOD, EROD or GST. All enzyme activities in treated groups were comparable to values for the controls after the 4-week recovery period. Some additional (non-GLP) measurements were performed on livers from rats in the control group and rats at the highest dose that involved assaying the hydroxylation of testosterone as an indicator of other CYP subtypes. Hydroxylation was slightly inhibited at the 16α- and 2α- positions in males at the highest dose, and slightly increased at the 6β- position in males and females at the highest dose.

There were no treatment-related findings on gross examination at autopsy. In the group at 500 mg/kg bw per day, the liver weights of female rats were significantly increased by 12%, but this difference had disappeared by the end of the recovery period. There was no effect on the weights of livers of male rats. There were tendencies for spleen weights to decrease in the treated groups, an effect that was statistically significant in males at 500 mg/kg bw (15%) and in females at 100 mg/kg bw (16%); however, the effect was not significant in females at 500 mg/kg bw (9%) and the effects had disappeared by the end of the recovery period. There were no histological findings that could have correlated with these effects on liver and spleen weights. Other organ weights were not affected.

The histological examination revealed treatment-related effects only in male and female rats at the highest dose, 500 mg/kg bw per day. There was slight hypertrophy of the centrilobular hepatocytes in which the cytoplasm had a fine granular structure consistent with increased enzyme activity in 6 out of 10 males and 2 out of 10 females. At the highest dose, but only in male rats, there was also increased severity of basophilic tubules in the renal cortex (Table 17). Both the hepatic and renal effects had resolved by the end of the recovery period. There were no other effects of treatment. The liver and kidney were confirmed as target organs for prothioconazole effects in rats after repeat dosing.

The NOAEL was 100 mg/kg bw per day on the basis of increased water consumption, decreased urinary volume, decreased spleen weights (males only), increased incidence and severity of basophilic tubules in the kidney (males only) and one mortality in females, possibly related to kidney failure, at 500 mg/kg bw per day. It seems reasonable that the increased liver weights (females only), hepatocytic hypertrophy (males and females) and changes in liver microsomal enzyme activities at 500 mg/kg bw per day were adaptive responses to treatment, rather than adverse effects, but this is not entirely clear given that the weight increases were in females, but the enzyme activity increases were greater in males. All of these findings had resolved by the end of a 4-week recovery period (Wirnitzer & Hartmann, 1999a).

Table 17. Incidence of treatment-related histopathological lesions in rats given prothioconazole by gavage for 14 weeks

Finding	Dose (mg/kg bw per day)							
	Males				Females			
	0	20	100	500	0	20	100	500
Liver[a]								
No. examined	10	10	10	10	10	10	10	10
Hypertrophy	0	0	0	6	0	0	0	2
Cytoplasmic change	0	0	0	6	0	0	0	2
Kidneys[a]								
No. examined	10	10	10	10	10	10	10	10
Basophilic tubules:[b]								
Grade 1	4	7	6	4	2	1	0	3
Grade 2	1	1	2	5	0	0	0	0
Total No. affected	5	8	8	9	2	1	0	3

From Wirnitzer & Hartmann (1999a)

[a] After the recovery period there were no rats with liver hypertrophy or cytoplasmic changes, and the incidence of basophilic tubules was similar in treated and control rats.

[b] All basophilic tubules in this study were graded as minimal (grade 1) or slight (grade 2) by the study pathologist.

Groups of 10 male and 10 female Wistar rats were given prothioconazole technical (purity, 98.5%) as a dermal application at a dose of 0, 100, 300 or 1000 mg/kg bw per day for 6 h per day for 28 or 29 days. Males received 22 applications over 28 days, females received 23 applications over 29 days (5 days per week for 3 weeks, then 7 days per week for the final week). The test material (a powder) was moistened with water and applied directly to the skin under a gauze pad and an occlusive dressing. The application site was washed with soap and water after each exposure period.

The state of health of the rats was checked and clinical signs were recorded daily. Dermal irritation was assessed daily (by the Draize method) and skin-fold thickness was measured with callipers at 3–4-day intervals. Eye examinations were conducted on all rats before dosing and on all surviving rats in the control group and those at 1000 mg/kg bw on day 27. Body weights were measured daily for dosing purposes and were recorded weekly. Food consumption was recorded weekly. Clinical examinations were performed outside the home cage before the dosing period and once per week thereafter. Blood samples were also taken on day 7 (shortly before the end of the 6-h exposure period) and levels of prothioconazole and prothioconazole-desthio were analysed in plasma. Laboratory investigations were carried out on all rats at the end of the treatment period. Rats were killed on study day 29 and were subjected to a detailed gross pathological examination. Selected organs were collected, weighed, and examined microscopically.

The doses were based on the results of a pilot study (not submitted) in which female rats received prothioconazole at a dose of 0 or 1000 mg/kg bw per day for 10 days during a 14-day period, during which no toxicologically significant effects were recorded.

Plasma concentrations of prothioconazole were below the LOQ (0.4 nmol/ml or 0.07 μg/ml) in all rats except for one male and one female in the group at 1000 mg/kg bw per day (1.3 and 1.2 nmol/ml respectively). Concentrations of prothioconazole-desthio in the blood were below the LOQ (0.4 nmol/ml or 0.06 μg/ml) in all rats.

There were no mortalities or clinical signs indicative of a treatment-related effect and there were no ocular effects. Body weights were not affected by treatment. There were isolated incidences of erythema in 1 out of 10 females at 100 mg/kg bw per day, none at 300 mg/kg bw per day and 2 out of 10 females at 1000 mg/kg bw per day. In the absence of a consistent effect, these findings were attributed to mechanical irritation at the application site due to the dosing procedure. There was no effect on skin thickness. Haematology and blood chemistry values were similar in all groups. There were no effects on organ weights and no treatment-related gross or microscopic findings.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Kroetlinger & Hartmann, 2000).

Dogs

Groups of four male and four female pure-bred beagle dogs were given prothioconazole (purity, 98.1–98.8%) at a dose of 0, 25, 100 or 300 mg/kg bw per day by gavage in 0.5% methylcellulose with 0.4% Tween 80 in deionized water, 5 days per week for 13 weeks. Additional control groups and groups at the highest dose were maintained for a further 8 weeks without treatment. Doses were selected on the basis of the results of a pilot study (not submitted) in which one male and one female per group received prothioconazole at a dose of 0, 10, 50, 200, 450 or 1000 mg/kg bw per day for 4 weeks. The accuracy, stability and homogeneity of the dosing solutions were confirmed by analysis. The state of health of the dogs was checked and recorded daily. Body weights and food consumption were assessed weekly. Physical examinations, including ophthalmoscopy, electrocardiography, blood-pressure assessments, rectal temperatures, thoracic auscultation and neurological assessments of behaviour, gait, posture and reflexes were performed before treatment and after 13 weeks of treatment. Haematology, blood chemistry (including T3, T4 and TSH) investigations and urine analysis were performed after approximately 1, 2 and 3 months of treatment and after 1 and 2 months of the recovery period. All dogs were subjected to gross pathological assessment after 13 or 21 weeks

(recovery groups), followed by microscopic examinations of selected tissues and organs. Additional samples of liver and kidney were taken for analysis of CYP and conjugation enzymes and for assay of testosterone hydroxylation. Concentrations of prothioconazole and prothioconazole-desthio were analysed in samples of liver and kidneys from dogs at the highest dose.

In the 4-week pilot study, there were indications of adverse effects on the liver (blood chemistry parameters and increased weights) and the kidneys (histopathological lesions) at ≥ 200 mg/kg bw per day.

Concentrations of prothioconazole and prothioconazole-desthio were measured in samples of liver and kidney from dogs at the highest dose after 13 weeks of treatment. Concentrations in the liver of female dogs (7.2 ± 8.5 nmol/g tissue; $n = 4$) were approximately three- to fourfold those in males (2.4 ± 2.0 nmol/g tissue; $n = 4$). Clearly, however, the variation was substantial and the difference between the sexes was almost entirely due to a single high value of 19.3 nmol/g tissue from one bitch. Concentrations of prothioconazole in the liver of males and females were approximately 10-fold the concentrations of prothioconazole-desthio. Concentrations of prothioconazole in the kidney of female dogs (2.1 ± 2.1 nmol/g tissue) were also approximately three- to fourfold those in males (0.6 ± 0.5 nmol/g tissue). Again, the effect of a single high value of 4.9 nmol/g tissue from the kidney of the female that also had a high value in the liver had a substantial effect on the difference between the sexes. The concentrations of the prothioconazole-desthio metabolite in the samples of kidney were below the LOQ.

The only mortality was that of a female in the recovery group, previously treated at the highest dose, which died on day 50 due to a dosing error. The only significant clinical signs noted were sporadic episodes of vomiting and increased salivation after dosing. These signs occurred in rats receiving prothioconazole and rats in the control group, with no dose–response relationship.

There was no effect on food consumption. Body-weight gains of male dogs at 300 mg/kg bw per day were lower than those of controls in the main group (–37%), but were similar to those of controls in the recovery group (–4%). In the absence of a consistent effect in both groups at 300 mg/kg bw per day, the lower body-weight gains in one group are of uncertain toxicological significance. Similar inconsistencies were observed in the females at the highest dose, where body-weight gains were lower than those of controls in the recovery group (–22%) but higher in the main group (+17%). Body weights were not affected by treatment at lower doses.

No effects of treatment were recorded on electrocardiograms, blood pressure assessments, rectal temperatures, thoracic auscultation or neurological assessments. Also, there were no treatment-related ocular changes. The main observations in haematology were lower erythrocyte counts and increased mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) in males at 100 and 300 mg/kg bw per day, but similar differences were also present before treatment, hence the significance of these findings is equivocal. There were no clear effects recorded on urine analysis parameters, though it was noted that the data from urine analysis showed marked variation.

A number of statistically significant differences were observed among several blood-chemistry parameters that were not related to dose, were similar to values before dosing, were within the range for historical controls, or did not persist over the various bleeding intervals. These changes were considered to be background or normal variations and not related to administration of prothioconazole. There were, however, several other blood-chemistry parameters related to hepatic or thyroid functions that might be considered to be affected by administration of prothioconazole in this study. Serum ALT activity was increased in females at 300 mg/kg bw per day after recovery by up to 550% (2-month sampling time) and, within this particular group, activity was increased at all sampling times (1, 2 and 3 months) during treatment. Although the values decreased during the 2-month recovery period, they remained significantly higher than those for the controls. In the other group of female dogs receiving prothioconazole at 300 mg/kg bw per day, the activity of this enzyme was increased by approximately twofold at the 2- and 3-month sampling times. The activity of AST was not increased

at 100 mg/kg bw per day. ALP activity was significantly increased in females in the recovery group at 300 mg/kg bw per day, but not in the non-recovery group. There was a partial return to normal levels during recovery. Since treatments of the non-recovery and recovery groups were apparently the same, the inconsistency in response was unexplained. There was no effect on AST activity. Gamma-glutamyltransferase activities were slightly increased in females at 300 mg/kg bw per day, but the observation was not reproducible in the two groups exposed at this dose.

Plasma T4 concentrations were consistently lower in females of the two groups at 300 mg/kg bw per day at the 2- and 3-month sampling times, and remained slightly lower than those of controls during the recovery period, though in some cases concentrations had also been lower before treatment. In male dogs, T4 concentrations was reduced in only one of the groups at 300 mg/kg bw per day and then only at the 2-month sampling time. Mean values for T4 concentrations were the same (2.0 µg/dl) in females of the groups at 25 and 100 mg/kg bw, but, because of the lower variation, this value was significantly lower than the control (2.9 µg/dl) only in the group at 25 mg/kg bw per day. Plasma TSH concentrations were not increased or decreased in any consistent manner after treatment.

At autopsy, the only findings considered to be treatment-related were multiple zones described as cysts on the surface of the kidney recorded in one out of four dogs in the main group and two out of four dogs in the recovery-group that had received a dose of 300 mg/kg bw per day. Absolute organ weights in treated dogs were similar to those of the controls, but increased relative (to body weight) organ weights were found for the liver, kidneys and thymus in females at 300 mg/kg bw per day and for the liver and kidneys in males at 300 mg/kg bw per day. Increased relative liver weights (11%) were also found in females at 100 mg/kg bw per day. Relative organ-weight increases were not observed in dogs at the end of the recovery period.

Treatment-related adverse histological findings were recorded for the kidneys of males and females at 100 and 300 mg/kg bw per day (see Table 18 for incidence and mean severity as assessed on a five-point scale). Males were affected to a greater extent than were females. Findings consisted of multifocal chronic interstitial fibrosis in the cortex, which often extended into the medulla. Foci adjacent to the capsular surface had been identified as cysts at autopsy. Minimal inflammatory cell infiltration (particularly lymphocytes) was present in most lesions. Compensatory hyperplastic change was present in some adjacent tubules. In some foci, crystalline material occurred in association with minimal haemorrhage and acute inflammation, or with chronic inflammation. Renal proximal tubular epithelial-cell swelling and dissolution with minimal pycnosis also occurred in three males at 300 mg/kg bw per day. Chronic inflammation remained in two males and one female in the recovery group 8 weeks after cessation of treatment.

Three treated females (one at 100 mg/kg bw per day and two at 300 mg/kg bw per day) had an increase in the number of polyovular follicles in the ovary compared with controls. However, these numbers were within the range for historical controls (0–50 combining pre-antral and/or antral follicles). There were no supporting observations of changes to reproductive function in these bitches, including no estrous-cycle aberrations (though most dogs were in a pre-maturation state), absolute ovarian weight or uterine morphology.

Focal or multifocal fibrosis of the lungs was observed, but there were no indications of a relationship with treatment. No histopathological observations were made in other organs that might have been considered to be related to treatment; in particular, there were no notable histopathological findings in the liver.

Hepatic microsomal enzyme activities were only marginally affected by treatment. EH activity in females was increased up to twofold at 100 and 300 mg/kg bw per day, while among males of the group at 300 mg/kg bw per day there were small reductions in the activities of 7-ethoxyresorufin-deethylase (EROD) and ALD. These small differences had resolved by the end of the recovery period. There were no consistent effects on the metabolism of testosterone. Enzyme activities in kidney samples were very low, but no obvious differences between control and treated dogs were identified.

Table 18. Incidence and severity of histopathological findings in the kidneys of dogs given prothioconazole by gavage for 13 weeks

Finding	No. of dogs affected (mean severity[a])							
	Dose (mg/kg bw per day)							
	Males				Females			
	0	25	100	300	0	25	100	300
No recovery								
No. of dogs examined	4	4	4	4	4	4		4
Cyst	0	0	0	1 (2.0)	0	0	0	0
Degeneration	0	0	0	3 (2.0)	0	0	0	0
Inflammation	0	0	0	0	0	0	1 (1.0)	0
Inflammation, acute	0	0	1 (1.0)	1 (1.0)	0	0	0	0
Inflammation, chronic	1 (1.0)	0	3 (2.0)	3 (2.3)	0	0	1 (3.0)	1 (1.0)
Mineralization	0	2 (2.0)	0	0	1 (1.0)	0	0	0
Debris	0	0	1 (2.0)	2 (1.5)	0	0	2 (2.0)	0
Lipidosis, glomerular	1 (1.0)	2 (1.0)	0	1 (1.0)	0	0	0	0
Recovery groups								
No. of dogs examined	4	0	0	4	4	0	0	3
Cyst	0	—	—	1 (2.0)	0	—	—	0
Degeneration	0	—	—	0	0	—	—	1 (3.0)
Inflammationok	0	—	—	0	0	—	—	0
Inflammation, acuteok	0	—	—	0	0	—	—	0
Inflammation, chronicok	0	—	—	2 (2.0)	0	—	—	1 (2.0)
Mineralization	0	—	—	0	0	—	—	0
Debris	0	—	—	0	0	—	—	0
Lipidosis, glomerular	0	—	—	0	1 (1.0)	—	—	0

From Jones & Stuart (2001a)

[a] Severity was graded from 1 (minimal) to 5 (severe).

Kidney was identified as the primary target organ for prothioconazole in dogs. The effects on the kidney were more evident in male dogs, in spite of the higher concentrations of prothioconazole in females, and had only partially resolved during the 8-week recovery period. There was also evidence of effects on the liver at 300 mg/kg bw per day, where relative liver weights were increased, and there was also an increase in plasma ALT activity that tended to persist throughout the recovery period, but there were no treatment-related histopathological findings.

The NOAEL was 25 mg/kg bw per day on the basis of histopathological changes in the kidneys at 100 mg/kg bw per day (Jones & Stuart, 2001a).

Groups of four male and four female pure-bred beagle dogs were given prothioconazole (purity, 98.1–98.8%) at a dose of 0, 5, 40 or 125 mg/kg bw per day by gavage in 0.5% methylcellulose with 0.4% Tween 80 in deionized water, 5 days per week for 52 weeks. Doses were selected on the basis of the 13-week study summarized above (Jones & Stuart, 2001a). The accuracy, stability and homogeneity of the dosing solutions were confirmed by analysis. The state of health of the dogs was

checked and recorded daily. Body weights and food consumption were assessed weekly. Physical examinations, including ophthalmoscopy, electrocardiography, blood-pressure assessments, rectal temperatures, thoracic auscultation and neurological assessments of behaviour, gait, posture and reflexes were performed before treatment and at the end of the study. Haematology, blood chemistry (including T3, T4 and TSH) investigations and urine analysis were performed after approximately 3, 6, 9 and 12 months of treatment. All dogs were subjected to gross pathological assessment after 52 weeks, followed by microscopic examinations of selected tissues and organs. Additional samples of liver were taken for analysis of CYP and conjugation enzymes.

There were no deaths. The only significant clinical signs noted were sporadic episodes of vomiting and increased salivation after dosing. These signs occurred in dogs in the treated and the control groups, with no dose–response relationship.

In female dogs at the highest dose, food consumption was lower than that of dogs in the control group throughout most of the study. Overall body-weight gain was lower than that of controls in the group at 125 mg/kg bw per day (14% and 42% lower in males and females, respectively), and was also marginally (11%) lower in males at 40 mg/kg bw per day. Lower body-weight gains started to become apparent after approximately 4 weeks of treatment. Body weights at termination in females at the highest dose were markedly lower than those in other groups of females.

No effects of treatment were recorded on electrocardiograms, blood-pressure assessments, rectal temperatures, thoracic auscultation or neurological assessments. Also, there were no treatment-related ocular changes.

Haematology parameters were similar in dogs receiving prothioconazole and in dogs in the control group at all time-points and at all doses. Similarly, there were no effects of treatment on urine-analysis parameters. The only consistent blood-chemistry findings were increased serum ALP activity in females at 40 and 125 mg/kg bw per day. The values were higher in these two groups than in controls at all time-points and were significantly so at 3, 6 and 12 months. Although ALP activities were also higher in males at these doses, the increases were not statistically significantly. T4 concentrations tended to be lower in males and females at 40 and 125 mg/kg bw per day, and were significantly so in males at 9 months (125 mg/kg bw per day) and 12 months (40 mg/kg bw per day) and in females at 3, 6 and 12 months (at both 40 and 125 mg/kg bw). There were no corresponding changes in T3 or TSH concentrations, hence the T4 changes are of uncertain toxicological significance.

The pattern of autopsy findings did not indicate any treatment-related effects. Analysis of organ-weight data revealed that there were no significant increases in the weights of any organs, including liver and kidney. Relative (to body) liver weights were significantly increased in male and female dogs at 125 mg/kg bw per day and relative (to body) kidney weights were significantly increased only in female dogs at 5 and 125 mg/kg bw per day, but not at 40 mg/kg bw per day.

Treatment-related histopathological changes were apparent in the liver and kidneys of males and females at 125 mg/kg bw per day (Table 19). The morphological changes in the kidney were characterized by minimal to mild focal to multifocal chronic inflammation of the renal cortex, often with extensions into the medulla. Minimal inflammatory cells, particularly lymphocytes, were also present. Adjacent tubules frequently showed compensatory hyperplastic changes and, in some foci, crystalline material was present. Inflammation occurred in some males at 40 and 125 mg/kg bw per day and in all females at 125 mg/kg bw per day. Crystalline material occurred in females at 40 and 125 mg/kg bw per day, and in one male at 125 mg/kg bw per day.

In the liver, pigmentation (which stained for iron and bile) was recorded in all females at the highest dose and one male at the highest dose, most prominently in the Kupffer cells. No hepatocellular hypertrophy was recorded in any group. The degree of pigmentation (compatible with iron) in the spleen was minimally increased in the spleen in females at 40 and 125 mg/kg bw per day. In the absence of haematological findings, the toxicological significance of this is uncertain. There were no notable histopathological findings in any other organs or tissues.

Table 19. Histopathological findings in the liver and kidneys of dogs given prothioconazole by gavage for 52 weeks

Finding	No. of dogs affected (mean severity[a])							
	Dose (mg/kg bw per day)							
	Males				Females			
	0	5	40	125	0	5	40	125
Kidney								
No. of dogs examined	4	4	4	4	4	4	4	4
Crystals	0	0	0	1 (1.0)	0	0	1 (1.0)	2 (1.0)
Cyst	1 (1.0)	1 (1.0)	0	0	0	0	0	0
Fibrosis	0	0	0	0	0	0	0	1 (1.0)
Hyperplasia	0	0	0	0	0	1 (1.0)	0	0
Inflammation, chronic	0	0	1 (2.0)	1 (3.0)	1 (2.0)	0	0	4 (1.5)
Inflammation, chronic active	0	0	1 (1.0)	0	1 (1.0)	0	0	0
Mineralization	0	0	0	1 (1.0)	1 (1.0)	1 (1.0)	0	0
Pigmentation	0	0	1 (3.0)	2 (1.5)	1 (1.0)	0	1 (2.0)	0
Lipidosis, glomerular	0	0	0	0	1 (1.0)	0	0	0
Mineralization, pelvis	0	1 (1.0)	0	0	0	1 (1.0)	0	0
Liver								
No. of dogs examined	4	4	4	4	4	4	4	4
Haematopoiesis, extramedullary	0	0	0	0	0	0	0	1 (1.0)
Kupffer cell aggregates	4 (1.0)	3 (1.0)	4 (1.0)	3 (1.3)	4 (1.3)	1 (2.0)	3 (1.0)	4 (1.0)
Pigmentation	0	0	0	1 (3.0)	0	0	0	4 (1.5)

From Jones & Stuart (2001b)

[a] Severity was graded from 1 (minimal) to 5 (severe).

There were no significant increases in the activities of any liver enzymes in liver sections taken at autopsy.

Kidney was identified as the primary target organ for prothioconazole in dogs, consistent with the findings of the 13-week study. There was also evidence of effects on the liver at 125 mg/kg bw per day, where relative liver weights were increased and there was also an increase in pigmented material.

The NOAEL was 5 mg/kg bw per day on the basis of histopathological changes in the kidneys at 40 mg/kg bw per day (Jones & Stuart, 2001b).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 60 male and 60 female CD-1 mice were given prothioconazole (purity, 98.2–98.8%) at a dose of 0, 10, 70 or 500 mg/kg bw per day by gavage in 0.5% Tylose for up to 80 weeks (18 months). The study complied OECD guideline 451 (1981).

Mortality was checked twice per day, clinical signs were recorded once daily and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly for the first 3 months and monthly thereafter.

Differential blood counts were performed on blood smears from mice in the control group and mice at the highest dose during week 78 (blood smears were also prepared from moribund mice, but no significant results were obtained). Surviving mice were killed after 18 months and subjected to a detailed autopsy. All mice found dead or killed in a moribund condition were also examined grossly and, if autolysis was considered to be not too advanced, they were examined microscopically. Specified organs of the mice were weighed (survivors at 18 months only) and specified tissues were collected, fixed and examined microscopically.

The accuracy, stability and homogeneity of the dosing solutions were confirmed by analysis. Survival was not affected by treatment. The numbers of deaths before 80 weeks were 9, 3, 7 and 14 (males) and 11, 9, 11 and 13 (females) at 0, 10, 70 and 500 mg/kg bw per day, respectively. There were no clinical signs considered to represent a specific response to treatment. Piloerection, pallor and poor general condition were noted at a higher incidence in mice at 70 and 500 mg/kg bw per day, but these observations were generally in mice shortly before unscheduled deaths or in old mice close to the end of the experiment and as such were not considered to be toxicologically significant.

Body-weight gains were statistically significantly reduced in males and females at 70 and 500 mg/kg bw per day. Lower body-weight gains started to become apparent after approximately 4-6 weeks of treatment. At 500 mg/kg bw per day, terminal body weights were approximately 9% and 13% lower than those of the controls in males and females, respectively. The corresponding terminal body-weight reductions at 70 mg/kg bw per day were 3% and 7%. Food consumption was similar between mice treated with prothioconazole and mice in the control group.

Differential blood counts at the end of the study revealed a significantly increased proportion of monocytes in males from 0.1% in the control group to 1.1% in the group at 500 mg/kg bw per day. There were also inconsistent effects on the incidence of atypical lymphocytes, which was significantly decreased in males (from 13.5% to 9.6%), but significantly increased in females (from 9.3% to 13.5%). Since these results were not consistent between sexes and were not accompanied by histopathological findings in haematopoetic organs (see below), these findings were not considered to be toxicologically significant.

Liver weights (absolute and relative to body weight) were increased in males and females at 70 and 500 mg/kg bw per day. The absolute liver weights were statistically significantly increased by 12.4% and 25.3% at 70 and 500 mg/kg bw per day, respectively, in male mice and by 20.6% at 500 mg/kg bw per day in female mice. Absolute kidney weights were lower by 20.5% and 15% at 500 mg/kg bw per day in male and female mice, respectively. Relative kidney weight was also depressed at this dose. Absolute (males and females) and relative (males only) heart weights were lower in all groups treated with prothioconazole, but the dose–response relationship was unclear. The decreases in absolute heart weight were 10.8%, 14.1% and 16.5% in males and 15.0%, 11.8% and 16.5% in females of the groups at 10, 70 and 500 mg/kg bw per day, respectively. The corresponding relative (to body weight) heart weights were 12.3%, 11.4% and 8.1% in males, while in females only the group at 10 mg/kg bw per day showed a significant relative heart-weight decrease, of 12.2%. Lower weights at the highest dose could be related to the lower terminal body weights, but not at the intermediate and lowest dose. In the absence of any notable histopathological lesions in the heart (see below), the lower heart weights are not considered to be toxicologically significant. Significantly lower absolute and relative epididymis and uterus weights at the highest dose were considered to be secondary to the lower terminal body weights.

Significant gross necropsy findings were increased incidence of distinct lobulation of the liver (males: controls, 0 out of 60; highest dose, 5 out of 60) and changes to the surface (males: controls, 0 out of 60; highest dose, 19 out of 60) and the colour of the kidneys (males: controls, 2 out of 60, highest dose, 7 out of 60; and females: controls, 4 out of 60; highest dose, 7 out of 60). The findings in the seminal vesicles (decreased incidence of discoloration from 12 out of 60 in the control group to 4 out of 60 at the highest dose) and uterus (decreased incidence of consistency change from 18

out of 60 in the control group to 4 out of 60 at the highest dose) were not considered to be treatment-related because the incidences of the correlating histopathological findings (glandular ectasia and cystic endometrial hyperplasia respectively) were not significantly different in the groups treated with prothioconazole and the control groups.

Treatment-related non-neoplastic histopathological findings in the liver consisted of centrilobular hepatocellular hypertrophy and fine granular eosinophilic change at statistically significantly higher incidences in males (48 out of 60) and females (33 out of 60) at 500 mg/kg bw per day and in males (31 out of 60) at 70 mg/kg bw per day. These kinds of effects were not recorded for any mice at lower doses or in the controls. In the kidneys, tubular degeneration and regeneration occurred at increased incidences in males (50 out of 60) and females (37 out of 60) at 500 mg/kg bw per day and in males (33 out of 60) at 70 mg/kg bw per day. Incidence in the control group was 23 out of 60 and 21 out of 60 in male and female mice, respectively. The incidence of renal subcapsular tubule degeneration with interstitial fibrosis was increased in males (34 out of 60) and females (27 out of 60) at 500 mg/kg bw per day. Incidences in all other groups ranged from 2 out of 60 to 6 out of 60. A decreased incidence of lymphoid hyperplasia in the mesenteric lymph nodes was noted in males from a control value of 6 out of 60 to 1 out of 60 in the groups at 70 and 500 mg/kg bw per day, but a decrease in the incidence of this finding is not considered to be a result of treatment. There were no notable histopathological findings in the heart, and the histopathological correlates of the gross necropsy findings (mentioned above) in the seminal vesicles and the uterus showed similar incidences to controls.

There was no increase in the number of tumour-bearing mice or total number of tumours in mice treated with prothioconazole (Table 20). Treated mice that died during the study did not show any overall increase in the incidence of tumours, suggesting that tumours were not associated with early death. Also, treatment was not associated with earlier occurrence of tumours.

The neoplastic findings by organ did not indicate any treatment-related effects. All neoplastic lesions occurring in liver, kidneys and all other organs and tissues in which possible treatment-related effects were noted are presented in Table 21, i.e. reproductive organs, spleen (haematological effects) and thyroid (thyroid hormone effects associated with liver enzyme induction). In particular, there was no increase in the incidence of neoplastic findings in either the liver or the kidneys, despite the treatment-related non-neoplastic effects in these organs. No neoplastic findings were recorded for either heart or seminal vesicles. There was a statistically significant decrease in the number of adenomas in the lung, but a decrease in the incidence of tumours was not considered to be toxicologically significant. The only other neoplastic findings which were statistically significant were the increased incidence of adenomas in Harderian glands of males at 70 mg/kg bw per day (8 out of 60); however, the incidence of this finding was similar in the group at the highest dose (2 out of 60) to that in the control group (1 out of 60) and in the absence of a dose–response relationship this finding was considered to be incidental.

The NOAEL was 10 mg/kg bw per day on the basis of reduced body-weight gain in male and female mice, liver-weight increases in male and female mice, hepatocellular-centrilobular hypertrophy and cytoplasmic change and renal-tubule degeneration and regeneration in male mice at 70 mg/kg bw per day. An additional observation made in all groups, including in mice at 10 mg/kg bw per day, was a reduction in heart weight, but there was no convincing dose–response relationship and no accompanying effect was observed microscopically; the Meeting therefore considered that this reduction in organ weight was not of toxicological significance (Schladt, 2001).

Rats

Groups of 80 male and 80 female Wistar rats received prothioconazole (purity, 98.8–99.4%) at a dose of 0, 5, 50 or 750 mg/kg bw per day by gavage for 53 weeks. The vehicle was an aqueous solution of 0.5% Tylose. The accuracy, stability and homogeneity of the dosing solutions were confirmed by analysis. Mortality and clinical signs were recorded daily and functional observational battery

Table 20. Incidences of benign and malignant tumours in mice given prothioconazole by gavage for up to 80 weeks

Tumour	Incidence							
	Dose (mg/kg bw per day)							
	Males				Females			
	0	10	70	500	0	10	70	500
Decedents								
No. of mice examined	9	3	7	14	11	9	11	13
No. with any tumour	4	2	5	4	6	5	6	5
No. with a benign tumour	0	0	3	1	1	1	1	1
No. with a malignant tumour	4	2	2	3	4	4	4	4
No. with benign and malignant tumours	0	0	0	0	1	0	1	0
No. with metastases	0	0	0	0	0	2	0	0
80 weeks								
No. of mice examined	51	57	53	46	49	51	49	47
No. with any tumour	15	23	22	12	20	12	9	11
No. with benign tumours	11	15	18	8	14	8	5	8
No. with malignant tumours	2	4	3	4	5	3	1	3
No. with benign and malignant tumours	2	4	1	0	1	1	3	0
No. with metastases	0	0	0	0	0	0	0	0
All mice combined								
No. of mice examined	60	60	60	60	60	60	60	60
No. with any tumour	19	25	27	16	26	17	15	16
No. with benign tumours	11	15	21	9	15	9	6	9
No. with malignant tumours	6	6	5	7	9	7	5	7
No. with benign and malignant tumours	2	4	1	0	2	1	4	0
No. with metastases	0	0	0	0	0	2	0	0

From Schladt (2001)

Table 21. Incidence of benign and malignant tumours in selected organs of mice given prothioconazole by gavage for up to 80 weeks

Tumour	Benign/ malignant	Incidence							
		Dose (mg/kg bw per day)							
		Males				Females			
		0	10	70	500	0	10	70	500
No. of mice examined									
Liver									
No. examined		60	60	60	60	60	60	60	60
Hepatocellular adenoma	B	6	7	5	2	0	0	1	1
Hepatocellular carcinoma	M	2	3	1	2	0	0	0	0
Haemangioma	B	3	0	1	0	0	1	1	0
Kidneys									
No. examined		60	59	60	60	60	60	60	60

Tumour	Benign/ malignant	Incidence							
		Dose (mg/kg bw per day)							
		Males				Females			
		0	10	70	500	0	10	70	500
Tubular cell adenoma	B	0	0	0	1	0	0	0	0
Epididymides									
No. examined		60	60	60	60	—	—	—	—
Sarcoma (NOS)	M	0	1	0	0				
Ovaries									
No. examined		—	—	—	—	60	60	60	60
Granulosa cell tumour	B	—	—	—	—	0	0	0	1
Luteoma	B	—	—	—	—	0	0	0	1
Uterus									
No. examined		—	—	—	—	60	60	60	60
Haemangioma	B	—	—	—	—	1	0	0	0
Leiomyoma	B	—	—	—	—	2	1	0	1
Stromal polyp	B	—	—	—	—	0	2	0	0
Adenomatous polyp	B	—	—	—	—	0	0	1	0
Stromal sarcoma	M	—	—	—	—	0	2	0	0
Schwannoma	M	—	—	—	—	1	0	0	1
Leiomyosarcoma	M	—	—	—	—	0	0	1	0
Spleen									
No. examined		60	60	59	60	60	60	60	60
Hemangioma	B	0	0	0	1	1	0	0	0
Thyroid gland									
No. examined		60	60	59	60	60	60	59	60
Follicular cell adenoma	B	0	0	0	0	0	1	0	0
Lungs									
No. examined		60	60	60	59	60	60	60	60
Alveolar/bronchiolar adenoma	B	3	7	9	2	10**	5	5	2*
Alveolar/bronchiolar carcinoma	M	2	2	2	2	1	0	2	1
Harderian glands									
No. examined		60	60	60	60	60	60	60	60
Adenoma	B	1	6	8*	2	3	1	2	5
Adenocarcinoma	M	0	1	0	0	0	0	0	0
All organs and tissues examined									
Total No. of mice examined		60	60	60	60	60	60	60	60
Total No. of tumours[a]		22	32	31	16	29	20	21	18
Total No. of benign tumours[a]		14	22	25	9	18	12	12	11
Total No. of malignant tumours[a]		8	10	6	7	11	8	9	7

From Schladt (2001)

NOS, not otherwise specified.

[a] Bilateral/multiple tumours at the same site and of the same type, classified as single tumour.

* $p < 0.05$;** $p < 0.01$ (one-sided Fisher's exact test).

(FOB) and grip-strength tests were performed on 10 males and 10 females per group at weeks 27 and 52. Individual body weights and food consumption were recorded weekly for the first 3 months and monthly thereafter. Eye examinations were conducted on all rats in the control group and in the group at 750 mg/kg bw per day and on the females at 50 mg/kg bw per day before the dosing period and at week 51. Laboratory investigations were carried out on 10 males and 10 females per group at 14, 27 and 52 weeks. Surviving rats were killed after 52 weeks and subjected to a detailed autopsy. All rats that were found dead or killed in a moribund condition were also examined. Specified organs of the rats were weighed and specified tissues were collected, fixed and examined microscopically.

There were three deaths of rats at 750 mg/kg bw per day that were not apparently related to errors in dosing or blood sampling. Two male rats died in weeks 40 and 51, respectively, and one female rat died in week 37. The study authors suggested that these deaths might be related to an increased severity of chronic progressive nephropathy, which was a consistent finding at this dose. Treatment-related increased salivation, increased urine excretion and bloody muzzle were observed in rats at 750 mg/kg bw per day. No treatment-related clinical signs were recorded in other groups. The FOB and grip-strength investigations did not identify any effects of treatment. Ophthalmoscopy revealed an increased incidence of water clefts in the anterior cortex of the lens in females at 750 mg/kg bw per day. This was described as an age-related lesion which is known to be a precursor of lens cataracts and can occur in rats of this age with a relatively wide variation in frequency.

Body-weight gains of male and female rats at 750 mg/kg bw per day were significantly reduced from week 13 of treatment. At termination, body weights in this group were up to 14% lower than controls. Body weights of rats in the groups at 5 and 50 mg/kg bw per day were comparable to those of the controls. Food consumption was similar in all groups. Hence, the lower body weights in rats at 750 mg/kg bw per day were not matched by lower food consumption. Water consumption was markedly increased in males and females at 750 mg/kg bw per day. Cumulative mean water intake over the whole study period was 84% higher in males and 45% higher in females. Water consumption was comparable in rats the control group and in groups of rats at 5 and 50 mg/kg bw per day.

Significant haematology findings were confined to rats in the group at the highest dose. Mean haemoglobin concentration (but no other erythrocyte parameters) was slightly reduced in males at 750 mg/kg bw per day at termination only. Platelet count was increased in males at 750 mg/kg bw per day at all time-points tested. Similar trends in haemoglobin concentration and platelet counts were seen in females at 750 mg/kg bw per day, but the differences were marginal. Leukocyte counts were higher in males and females at 750 mg/kg bw per day (mostly not statistically significant) than in the controls at most time-points, but there were no differences in the subpopulations (differential leukocyte counts). A lower thromboplastin time in males at 750 mg/kg bw per day at week 14 only was dismissed since the effect did not persist at other time-points and the values for individual animals in the group were within normal ranges.

Clinical chemistry investigations revealed a number of effects considered to be related to treatment at 750 mg/kg bw per day. ALP activity (but not ALT or AST) was statistically significantly increased in males (week 14) and females (weeks 27 and 52) at 750 mg/kg bw per day. Plasma concentrations of urea, creatinine, bilirubin, cholesterol and triglycerides were all increased in males and females at 750 mg/kg bw per day at most time-points. Plasma protein concentration was reduced in males at 750 mg/kg bw per day at the end of the study. Each of these clinical chemistry parameters were similar to controls at 50 and 5 mg/kg bw per day. There was no effect of treatment on electrolytes in any group.

Concentrations of T4 in plasma were consistently lower in males and females at 750 mg/kg bw per day at all time-points. The only similar effect on T3 concentrations occurred in males at 750 mg/kg bw per day at the end of the study. TSH concentrations (analysed at weeks 14 and 52 only) were lower than controls at 750 mg/kg bw per day in males at week 14 and in females at weeks 14 and 52 (more marked effect at week 14).

Treatment-related effects on urine-analysis parameters were also recorded at 750 mg/kg bw per day. These included increased urinary volume and correspondingly reduced urinary density and lower urine pH. Males were affected to a greater extent than females. Examination of sediment revealed yellow-brown crystalloid ball-shaped structures in most rats at the highest dose at week 27 and in all rats at the highest dose at the end of the study. There were no similar effects at 5 or 50 mg/kg bw per day.

There were a number of increased relative (to body) organ-weight changes at 750 mg/kg bw per day that could be largely attributed to the adverse body-weight effects in these rats. However, significant relative increases in kidney weight of 17% and 10% in male and female rats, respectively were consistent with the toxic effects on this organ in other studies and smaller, non-significant increases in relative kidney weight were observed in male rats of the groups at 5 and 50 mg/kg bw per day. In addition, significant increases were observed in both absolute and relative (to body) liver weights of 14% and 29%, respectively, in females of the group at 750 mg/kg bw per day.

Treatment-related findings at autopsy of rats at 750 mg/kg bw per day were increased incidences of caecal dilations (males, 3 out of 20; females, 12 out of 20), stomach covering (males, 2 out of 20; females, 3 out of 20), cysts in mesenterial lymph nodes (males, 3 out of 20; females, 1 out of 20) and changes of kidney surface (males, 17 out of 20; females, 5 out of 20). Treatment-related microscopic findings, mainly in the liver, kidneys and urinary bladder were recorded in males and females at 750 mg/kg bw per day (Table 22). The cytoplasmic change in hepatocytes was described as a granular appearance (known to be indicative of liver-enzyme induction) and occurred with a similar incidence and severity in males and females. There was also a slightly increased incidence of bile-duct hyperplasia in females (one female was markedly affected), but it is uncertain whether this was a treatment-induced effect. In males there was little evidence that this effect was treatment-related. Chronic progressive nephropathy occurred in almost all rats, particularly males, but there was a clear dose–response relationship in the severity of the lesions, with grades 3 and 4 (highest) combined occurring in 19 out of 20 males and 10 out of 20 females of the group at 750 mg/kg bw per day. is the study authors considered that there was no exacerbation of chronic progressive nephropathy at any lower dose in males or females. This microscopic finding correlated with the gross finding of roughened kidney surfaces and the overall renal pathology correlated with the results of urine analysis.

Of the other notable microscopic findings, some of the cysts identified in mesenteric lymph nodes at autopsy were described as sinus dilatations and the stomach-lining changes noted at autopsy were described as an increase in mucus. No histopathological correlate was found for either the dilation of the caecum or the ophthalmoscopic observation of water clefts in the eye lens. A total of 10 neoplasms were found in this study, but these were spread across the groups (including five in the control group) and affected a diverse range of organs and tissues. No neoplastic findings were considered to be related to treatment.

The NOAEL was 50 mg/kg bw per day, on the basis of liver and kidney effects and reduced body weights at 750 mg/kg bw per day (Wirnitzer & Popp, 2000).

Groups of 50 male and 50 female Wistar rats were given prothioconazole (purity, 98.5–99.1%) at a planned dose of 0, 5, 50 or 750 mg/kg bw per day by gavage in 0.5% Tylose for up to 105 weeks. The highest dose was reduced to 500 mg/kg bw per day in males from week 84 and to 625 mg/kg bw per day in females from week 56. The study complied with or exceeded the requirements of OECD guideline 451 (1981).

Mortality was checked twice per day, clinical signs were recorded once daily and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly for the first 4 months and monthly thereafter. Eye examinations were conducted on all rats before the dosing period and at weeks 51 and 104. Laboratory investigations were carried out on 10 males and 10 females per group at 53, 79 and 105 weeks. Surviving rats were killed after 104 weeks and subjected to a detailed autopsy. All rats found dead or killed in a moribund condition

Table 22. Histopathology findings in the liver, kidney, urinary bladder and lymph nodes of rats treated with prothioconazole by gavage for 52 weeks

Finding	Incidence							
	Dose (mg/kg bw per day)							
	Male				Female			
	0	5	50	750	0	5	50	750
Liver								
No. of rats examined	20	20	20	20	20	20	20	20
Cytoplasmic change	0	0	0	11	0	0	0	15
Grade 1	0	0	0	5	0	0	0	8
Grade 2	0	0	0	6	0	0	0	7
Bile-duct hyperplasia	4	8	1	1	2	2	0	5
Grade 1	4	8	1	0	2	2	0	4
Grade 2	0	0	0	1	0	0	0	0
Grade 3	0	0	0	0	0	0	0	1
Kidney								
No. of rats examined	20	20	20	20	20	20	20	20
Chronic progressive nephropathy	19	20	20	20	10	15	12	20
Grade 1	11	11	11	0	9	14	9	1
Grade 2	6	5	6	1	1	1	2	9
Grade 3	2	3	2	7	0	0	1	6
Grade 4	0	1	1	12	0	0	0	4
Urinary bladder								
No. of rats examined	20	20	20	20	20	20	20	20
Simple hyperplasia	1	1	1	14	0	0	0	12
Focal inflammatory infiltration	9	5	5	19	2	0	4	11
Mesenteric lymph node								
No. of rats examined	20	20	20	20	20	20	20	20
Sinus dilatation	0	0	0	4	0	0	0	1

From Wirnitzer & Popp (2000)

were also examined grossly and microscopically. Specified organs of the rats were weighed (survivors at week 105 only) and specified tissues were collected, fixed and examined microscopically.

The accuracy, stability and homogeneity of the dosing solutions were confirmed by analysis. Mortality was statistically significantly increased in male rats at the highest dose, and remained higher than that in the controls even after the reduction in the highest dose. Mortality was also higher in females at the highest dose until the dose was lowered to 625 mg/kg bw per day, after which the mortality rate was similar to that in the controls. The elevated mortality rate in males at the highest dose meant that survival in this group was less than 50% at week 94. Survival at termination in all other treated groups was similar to that in the controls.

Treatment-related clinical signs were recorded for male and female rats at the highest dose, consisting of increased incidences of emaciation, increased urine volume and poor general condition. Pallor and bloody muzzle were also recorded in males. The only notable clinical sign in rats given lower doses was increased urine volume in males at 50 mg/kg bw per day. Ophthalmoscopy revealed an increased incidence of water clefts in the anterior cortex of the lens in rats at the highest dose (in

females after 1 year, but in males after 2 years). The incidences were, however, within the range for historical controls for the laboratory and were considered to be secondary to the poor condition of the rats at the highest dose.

Body-weight gains of males and females at the highest dose were significantly lower than those of the controls, such that body weights in this group towards the end of the study were reduced by up to 20%. Differences from controls were statistically significantly reduced at 3 months of treatment in males and at 6 months of treatment in females. Group mean body weights in males declined from week 78 onwards and the body-weight decline was not reversed by lowering the highest dose. Body weights of rats in the groups at 5 and 50 mg/kg bw per day were comparable to those of the controls.

Food and water consumption were increased at the highest dose in males and females. Cumulative food consumption (relative to body weight) was approximately 15% higher than that of the controls i.e. despite having lower body weights than the controls, the group of rats at the highest dose consumed more food than did the controls. Cumulative water consumption of males at the highest dose was twofold that of the controls and approximately 50% higher than that of the controls in females at the highest dose. No effects were seen on food or water consumption at lower doses.

Erythrocyte counts, haemoglobin concentration and values for erythrocyte volume fraction were frequently statistically significantly depressed in males and females at 750 mg/kg bw per day at all sampling intervals, except for females at 105 weeks. Increased platelet, neutrophil and leukocyte counts were recorded in males (but not females) at 750 mg/kg bw per day and increased platelet counts were also recorded in males at 50 mg/kg bw per day. There were no notable haematology findings in the groups at lower doses.

There were a number of treatment-related effects on blood chemistry parameters of rats at 750 mg/kg bw per day. There were statistically significant reductions in ALT activity and increases in ALP in males and females. In males only, concentrations of glucose, protein and albumin were slightly reduced, urea and creatinine were markedly increased and cholesterol was slightly increased. Calcium and phosphate-ion concentrations also tended to be increased in rats at the highest dose. The only notable effect at lower doses was increased ALP activity in females at 50 mg/kg bw per day at all sampling times.

The concentration of T4 was statistically significantly reduced in plasma of rats at the highest dose for females at weeks 53, 79 and 105, and for males at weeks 79 and 105; it was also reduced at 50 mg/kg bw per day in females at weeks 105 and at 50 and 5 mg/kg bw per day in males at week 105. T3 was reduced in females at the highest dose at week 53. An increase in TSH that might be interpreted as a response to the changes in T4 was observed only at the highest dose in females at weeks 53 and in males at week 79. These sampling times, however, could already be too late to register such a response.

Treatment-related urine-analysis findings in rats at the highest dose comprised increased urinary volume from around 3–6 ml in the control group and groups at lower doses to 13–18 ml at weeks 53, 79 and 105 in males and females. These increases were accompanied by decreases in urine density, as might be anticipated. Males were affected to a greater extent than females. Yellow-brown crystalloid structures in urine sediment were also recorded in rats at the highest dose at week 53. The crystals were described as being insoluble in 5N NaOH, 30% acetic acid or ethanol, but soluble in 4N HCl. Urinary pH was significantly reduced in males at the highest dose at all three sampling times by about 0.7–1.0 pH units, in comparison with the controls. It was not clear that there was any effect on the pH of urine from females.

A number of organ-weight changes in the group at the highest dose were attributed to the adverse effects on body weights in these rats. There were statistically significant increases in relative (to body) weights of liver (males, 25%; females, 26%) and kidney (males 30%; females, 11%) at the highest dose. The absolute weights of these organs were not significantly altered by treatment.

At autopsy, some findings were recorded at higher incidences in rats in the group at the highest dose, particularly in males. These findings were: discoloured areas in the lungs, liver and stomach, kidney-surface changes (including cysts and discoloration), thickened urinary-bladder wall, oedematous salivary glands, consistency changes or flaccid testes, seminal vesicles reduced in size, content change in the rectum, content change and dilation in the caecum and dilation of the pancreas. Kidney-surface changes and discoloured areas in the lungs were also recorded at higher incidences in male rats (and at increased but somewhat lower incidences in females) at 50mg/kg bw per day. Liver discoloration was also recorded for males at 50 and 5 mg/kg bw per day. No other findings on gross examination at autopsy were considered to be significant.

There were treatment-related microscopic findings in the liver, kidneys and urinary bladder. Increased incidences or severity of some findings were recorded in males and females at the highest dose, and also in males at 50 mg/kg bw per day. The incidences of centrilobular hepatocellular hypertrophy with cytoplasmic change were increased in both male (42%) and female (66%) rats of the group at the highest dose and also in males (20%) of the group at 50 mg/kg bw per day, compared with 0% and 2% in males and females in the control group. Eosinophilic or clear-cell foci with cytoplasmic change were increased in males (18%) and females (32%) in the group at the highest dose, there being no differences at lower doses compared with the controls. Chronic progressive nephropathy was common in males and females, but there was a dose-related increase in the mean severity scores, particularly in males, from 2.5 in the controls to 2.7, 3.0 and 4.4 in the groups at 5, 50 and 750/500 mg/kg bw per day, respectively. The corresponding mean severity scores in females were 1.9, 1.7, 1.8 and 2.2. Among males at the highest dose, there was a high incidence of chronic progressive nephropathy in rats dying before termination, but a lower incidence than controls amongst rats that survived to termination; this indicates that the increased mortality was related to the renal disease. There were also increased incidences of transitional-cell hyperplasia in the urinary bladder (16% in males and females) at the highest dose, accompanied by inflammation in 8% of males and 4% of females. The study authors considered this to be a regenerative process in response to mechanical irritation and abrasion from the crystalloid bodies observed in urine sediment.

A number of other lesions occurred at an increased incidence in males at the highest dose; these lesions were considered to be secondary to the poor general condition of these rats (the highest incidence of these findings occurred in intercurrent deaths); however, none of these microscopic findings was increased in female rats. The increased findings in males were: tubular atrophy in the testes, oligospermia or aspermia in the epididymides, atrophy of seminal vesicles and prostate gland, increased aspiration pneumonia, increased arteritis and periarteritis in the pancreas and increased inflammation in the nasal cavity. Diffuse hyperplasia of the parathyroid glands occurred in 8%, 8%, 10% and 22% of the male rats in the groups at 0, 5, 50 and 750/500 mg/kg bw per day, respectively. These lesions were considered to be secondary to increased severity of chronic renal nephropathy. In females at the highest dose there was a decreased incidence of pressure atrophy in the brain to 2% from 14% in the controls. This effect was most likely due to a lower incidence of pituitary neoplasms. Notably, there were no significant histopathological findings in the thyroids, despite the changes in concentrations of thyroid hormones recorded throughout the study; C-cell hyperplasia was the most common lesion and occurred at a higher frequency in the control group than in the group at the highest dose.

There was no increase in the number of tumour-bearing rats or total number of tumours in treated rats (Table 23). The lower incidence of neoplastic findings in the group of male rats at the highest dose reflects the higher early mortality in this group. There were no treatment-related effects on the nature, incidence or time of discovery of any tumour type in treated rats. No treatment-related increases are apparent when premature decedents and rats killed at the end of study are examined separately, nor when benign and malignant tumours are considered separately. Treated rats which died during the study did not show any overall increase in the incidence of tumours, indicating that tumours were not associated with early death. The relationship of tumour incidence to time did not suggest that treatment with prothioconazole was associated with earlier occurrence of tumours.

Treatment-related lesions were discovered microscopically in a number of organs (see above), and the incidences of tumours in the affected organs are detailed in Table 24. No notable increases in the incidence of tumours were recorded in any of these organs and tissues and there were no neoplastic findings in the parathyroid glands.

The NOAEL was 5 mg/kg bw per day on the basis of increased platelet count in males, increased ALP activity at all sampling times in females, increased incidence of centrilobular hepatocellular hypertrophy with cytoplasmic changes in males and increased severity of chronic progressive nephropathy in males at 50 mg/kg bw per day. Some additional observations were made at 5 mg/kg bw per day, but the Meeting did not consider these to be of toxicological significance. These observations were discoloration of the liver in males, but unaccompanied by any histological changes, and a

Table 23. Incidence of benign and malignant tumours in rats given prothioconazole by gavage for up to 2 years

Parameter	Dose (mg/kg bw per day)							
	Males				Females			
	0	5	50	750/500	0	10	70	750/625
Premature decedents								
No. of rats examined	19	18	17	37	23	18	15	23
No. with any tumour	11	13	9	11	21	15	11	14
No. with a benign tumour	6	5	4	8	15	9	8	8
No. with a malignant tumour	4	6	5	0	4	4	1	5
No. with benign and malignant tumours	1	2	0	3	2	2	2	1
106 weeks								
No. of rats examined	31	32	33	13	27	32	35	27
No. with any tumour	14	16	19	3	23	25	26	20
No. with a benign tumour	11	14	15	3	16	20	22	15
No. with a malignant tumour	1	1	1	0	2	2	0	2
No. with benign and malignant tumours	2	1	3	0	5	3	4	3
All rats combined								
No. of rats examined	50	50	50	50	50	50	50	50
No. with any tumour	25	29	28	14	44	40	37	34
No. with a benign tumour	17	19	19	11	31	29	30	23
No. with with a malignant tumour	5	7	6	0	6	6	1	7
No. with benign and malignant tumours	3	3	3	3	7	5	6	4
Time of discovery of tumours								
Weeks 1–13	0	0	0	0	0	0	0	0
Weeks 14–26	0	0	0	0	1	0	0	1
Weeks 27–39	0	0	0	0	0	0	0	2
Weeks 40–52	0	1	0	0	1	1	0	1
Weeks 53–65	0	0	1	0	3	2	3	1
Weeks 66–78	0	0	1	1	1	3	1	0
Weeks 79–91	1	4	4	3	6	3	3	3
Weeks 92–104	7	8	2	7	7	4	3	2
Weeks 105–107	17	16	20	3	25	27	27	24

From Wirnitzer & Hartmann (2001)

reduction in T4 concentrations in females only at week 105, with no corresponding change in plasma TSH concentrations (Wirnitzer & Hartmann, 2001).

Table 24. Incidences of benign and malignant tumours in selected organs from rats given prothioconazole by gavage for up to 2 years

Tumour	Benign/ malignant	Incidence of tumours							
		Dose (mg/kg bw per day)							
		Males				Females			
		0	5	50	750/500	0	10	70	750/625
Liver									
No. of rats examined		50	50	50	50	49	50	50	50
Hepatocellular carcinoma	M	1	0	0	0	0	0	0	0
Cholangiocarcinoma	M	0	0	2	0	0	0	2	0
Bile duct[a]									
No. of rats examined		0	1	0	1	0	0	0	0
Adenocarcinoma	M	—	1	—	0	—	—	—	—
Kidneys									
No. of rats examined		50	50	50	50	49	50	50	50
Lipomatous tumour	B	0	1	1	0	0	0	0	0
Tubule-cell adenoma	B	0	1	0	0	0	0	0	0
Urinary bladder									
No. of rats examined		50	50	50	50	49	50	50	50
Transitional-cell papilloma	B	0	0	0	1	0	0	0	0
Nose									
No. of rats examined		50	50	50	50	49	50	50	50
Adenocarcinoma, skirrhous	M	1	0	0	0	0	0	0	0
Pancreas									
No. of rats examined		50	50	50	50	49	50	50	50
Islet-cell adenoma	B	0	2	1	0	0	1	0	1
Testes									
No. of rats examined		50	50	50	50	0	0	0	0
Leydig-cell tumour	B	3	3	3	2	—	—	—	—
Pituitary gland									
No. of rats examined		49	50	50	50	49	50	50	50
Pars distalis adenoma	B	9	12	12	6	20	20	23	13
Thyroid gland									
No. of rats examined		50	50	50	50	49	50	50	50
C-cell adenoma	B	3	4	4	2	3	6	3	2
Follicular cell carcinoma	M	1	0	0	0	0	0	0	0

From Wirnitzer & Hartmann (2001)

B, benign; M, malignant.

[a] Extrahepatic.

2.4 Genotoxicity

Prothioconazole was tested for genotoxicity in a range of assays, both in vitro and in vivo (Table 25). There was no evidence for induction of gene mutation in *S. typhimurium* in vitro (Herbold, 1996a). The dose-range that was used extended into a range that was bacteriotoxic for this compound. No significant response was observed in a single study for mutations at the *Hprt* locus in Chinese hamster lung V79 cells in vitro, after exposure for 5 h to prothioconazole at concentrations of up to 175 µg/ml, or 200 µg/ml in the absence or presence of metabolic activation, respectively (Brendler-Schwaab, 1996). Thus, prothioconazole did not induce gene mutations in either bacterial cells or cultured mammalian cells.

A single study for the induction of chromosomal aberration in vitro was conducted in Chinese hamster lung V79 cells. Exposures were for either 18 h or 30 h and there were significant increases in the frequencies of cells with aberrations (excluding gaps) at both treatment times and in the presence and absence of metabolic activation. The clastogenic effect was reproducible in independent experiments (Herbold, 1996b). Particularly significant were increased frequencies of cells with exchanges. These aberrations involve at least two chromosomal breaks within a cell and the ensuing daughter cells are likely to survive, while cells with chromosomal breaks not resulting in re-joining are not likely to survive once they have divided.

In a test for genotoxicity assessed by the induction of unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes exposed for 16 h to prothioconazole at concentrations of up to 20 µg/ml, equivocal results were obtained. There was no attempt to resolve the uncertainty in vitro (Brendler-Schwaab, 1998). However, in a study in vivo, there was no indication of UDS in the liver cells of rats treated with prothioconazole at a dose of 5000 mg/kg bw by gavage. Cells were examined 16 h after dosing (Brendler-Schwaab, 1999a).

In two studies of clastogenicity and aneugenicity, micronucleus formation was examined in bone-marrow cells of NMRI mice. In the first, groups of five male and five female mice were given prothioconazole as a single dose at 0 or 250 mg/kg bw by intraperitoneal injection and bone-marrow cells were sampled after 16 h, 24 h and 48 h. In the second study, groups of 10 male NMRI mice were given prothioconazole at a dose of 0, 50, 100 or 200 mg/kg bw by intraperitoneal injection on two occasions separated by 24 h, and bone-marrow cells were sampled after 24 h. No increases in the frequency of micronucleated polychromatic erythrocytes were observed in any group receiving prothioconazole, although there were clear signs of toxicity at these doses. Large increases in the frequency of micronucleus formation were observed in both of the groups acting as positive controls (Herbold, 1996c; Herbold, 2003).

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a pilot study to determine doses for a multigeneration study, groups of 10 male and 10 female Wistar rats were given prothioconazole (purity, 98.1–98.8%) at a dose of 0, 10, 100, 250 or 500 mg/kg bw per day by gavage in 0.5% aqueous methylcellulose/Tween 80 continuously, except during parturition, from 4 weeks before mating until weaning of offspring at postnatal day 21. Investigations made included body weights, food consumption and clinical signs in adults, litter parameters and pup weights and clinical signs during lactation (litter size was reduced to four males and four females per litter on day 4). Rats were killed when pups reached postnatal day 21. Gross necropsy was carried out for adults and weights of liver, kidneys, adrenals, thyroid, testes, uterus and ovaries were recorded.

Table 25. Results of studies of genotoxicity with prothioconazole

End-point	Test object	Concentration/dose[a] (LED/HID)	Purity (%)	Result	Reference
In vitro					
Gene mutation	*S. typhimurium* strains TA100, TA1535, TA1537, TA98; TA102, standard plate test and pre-incubation	500 µg/plate ± S9	99.5	Negative	Herbold (1996a)
Gene mutation	Chinese hamster lung V79 cells *Hprt* locus	175 µg/ml − S9 200 µg/ml + S9	99.8	Negative	Brendler-Schwaab (1996)
Chromosomal aberration	Chinese hamster lung V79 cells	50 µg/ml − S9 150 µg/ml + S9	99.8	Positive ± S9	Herbold (1996b)
Unscheduled DNA synthesis	Rat hepatocytes	20 µg/ml	99.7	Equivocal	Brendler-Schwaab (1998)
In vivo					
Unscheduled DNA synthesis	Male Wistar rat, hepatocytes	5000 mg/kg bw × 1, 16 h after dosing	99.5	Negative	Brendler-Schwaab (1999a)
Micronucleus formation	Male and female NMRI mice, bone-marrow cells	250 mg/kg bw × 1 (intraperitoneal), 16, 24 and 48 h after dosing	99.9	Negative	Herbold (1996c)
Micronucleus formation	Male and female NMRI mice, bone-marrow cells	200 mg/kg bw × 2 (intraperitoneal), 24 h after second dose	95.7	Negative	Herbold (2003)

S9, 9000 × *g* supernatant from rat livers.

The accuracy, stability and homogeneity of the dosing solutions were confirmed by analysis.

There were no parental deaths during the study and treatment-related clinical signs were confined to urine staining of the fur in one male and three females at 500 mg/kg bw per day before and during mating phases and in two females at 500 mg/kg bw per day during gestation. There were no other effects on adults and no treatment-related effects on reproductive parameters at any dose. There were no treatment-related clinical signs amongst pups during the postnatal period, and no significant effect on litter parameters (pup deaths) or pup weight during lactation (pup weights were slightly lower than those of the controls at the highest dose, but there were no statistically significant differences). Gross necropsy of pups did not reveal any notable findings.

The NOAEL was 500 mg/kg bw per day, the highest dose tested (Astroff, 1999).

In the subsequent multigeneration study, groups of 30 male and 30 female Wistar rats were given prothioconazole (purity, 98.1–98.8%) at actual doses of 0, 9.7, 95.6 or 726 mg/kg bw per day by gavage in 0.5% aqueous methylcellulose/Tween 80 continuously from 10 weeks before mating until weaning of the F_1 generation pups, except that dams were not dosed from day 21 of gestation until the completion of parturition. Selected F_1 progeny were then similarly treated until weaning of F_2-generation pups. Observations made included body weights, food consumption (not during mating period), clinical signs and litter parameters. Litters were culled to four males and four females per litter on postnatal day 4. The estrous cycle was assessed over a 3-week premating period in all females (F_0 and F_1 generations). The day of vaginal opening and preputial separation were recorded in F_1 rats. Anogenital distance was measured on the day of birth for all F_2 progeny.

All rats were subjected to autopsy at termination and selected organs weighed. Terminal estrous cycle stage was assessed. Uteri were examined for implantation sites. One testis and one epididymis from all F_0 and F_1 males were taken for sperm counts (homogenization-resistant spermatids and cauda epididymal sperm reserves respectively), morphology (F_1 generation only) and motility. A quantitative evaluation of the ovaries for pre-antral follicles, antral follicles and corpora lutea was performed on 10 parental females per generation from the groups at 0 and 726 mg/kg bw per day. From non-selected F_1 pups and all F_2 pups, brain, spleen and thymus were weighed from one male and one female pup per litter. The following organs and tissues from adults in the control group and adults at the highest dose (F_0 and F_1) were examined microscopically: cervix, epididymis (caput, corpus and cauda), gross lesions, adrenal glands, liver, ovaries, pituitary, prostate, testicles, seminal vesicles/coagulating gland, uterus, oviducts and vagina. Reproductive organs of any rats suspected of reduced fertility were also examined microscopically. The study was performed in anticipation of OECD guideline 416 (2001) and met or exceeded the requirements of that guideline except that in adult rats thyroids were not weighed. Morphological examination of sperm samples for the F_0 generation was not performed.

The accuracy of dosing solutions was determined at regular intervals by analysis, while the homogeneity and stability of dosing solutions had been established in the previous pilot study.

The only notable clinical signs were urine stains and salivation before dosing in adult males and females at the highest dose from both generations. There were no clinical signs in rats from groups at lower doses or in pups.

Body-weight gains in males of the F_0 generation at the highest dose during the pre-mating period were significantly lower than those of the controls by a maximum of 7%. Food consumption was increased by up to 19% in rats of this group; hence there was decreased efficiency of food use. There were no similar effects in females of the F_0 generation. In rats of the F_1 generation at the highest dose, body weights were initially lower than those of the controls by 7–17% and this difference was maintained during the F_1 pre-mating period. Since food consumption in these rats was increased by up to 28% over this period, efficiency of food use was also decreased. Body weights of F_1 males of

the group at 95.6 mg/kg bw per day were also significantly lower than those of the controls by up to 8%, but without an accompanying effect on food consumption. Among females of both generations at the highest dose, there was also a slight decrease in body-weight gain during gestation and a slight decrease in food consumption during lactation. There were no other notable effects on body weights or food consumption. There were no notable gross necropsy findings in adults. Significant organ-weight changes in adult rats were increased absolute and relative liver weights in male and females at 726 mg/kg bw per day in the F_0 and F_1 generations; in males at 95.6 mg/kg bw per day in the F_0 and F_1 generations; and in females at 95.6 mg/kg bw per day in the F_1 generation, while absolute and relative kidney weights were increased in males at 726 mg/kg bw per day, in the F_0 and F_1 generations; and absolute and relative thymus weights were reduce in females at 95.6 and 726 mg/kg bw per day in the F_0 generation.

Microscopic examination revealed an increased incidence of hepatocytomegaly in males (F_0 and F_1) and females (F_1 only) at 726 mg/kg bw per day. There was also an increased incidence of multifocal cortical nephrosis in males and in females of F_0 and F_1 generations at 726 mg/kg bw per day. The parental toxicity at 726 mg/kg bw per day was considered to be very high, even life-threatening, primarily on the basis of the kidney dysfunction and resulting dehydration. Similar findings had been made in other studies of prothioconazole in this rat strain. Thus, 500 mg/kg bw per day given to pregnant rats caused a clearly increased mean water intake (+24%) as well as dehydration/rough coat in one out of 12 dams. A dose of 1000 mg/kg bw per day caused a markedly increased mean water intake (+37%) and dehydration/rough coat/death in 25% of the pregnant rats (see below). Furthermore, markedly increased water intake, kidney damage and isolated deaths (possibly related to kidney failure) were observed in a 13-week study of toxicity in rats at 500 mg/kg bw and in a 1-year study of toxicity in rats at 750 mg/kg bw (two males in weeks 40 and 51; one female in week 37). Finally, a high mortality rate, which was obviously related to kidney failure, was observed in males and females in the 2-year study of toxicity and carcinogenicity in rats at doses of 500–750 mg/kg bw.

A treatment-related decrease in the number of estrous cycles and concomitant increase in the cycle length (cycles per 14 days) occurred at 726 mg/kg bw per day in both the F_0 generation (control group, 3.4; highest dose, 2.7) and F_1 generation (control group, 3.6; highest dose, 3.1). The effect was not apparent at lower doses. There were no treatment-related effects at any dose on the mating, fertility and gestation indices. However, at 726 mg/kg bw per day there was a non-statistically significant greater number of days to insemination in the F_1 generation (control group, 2.4 days; highest dose, 3.8 days) and in both generations there were lower mean numbers of implantation sites (F_0 control group, 11.8; F_0 highest dose, 10.8; F_1 control group, 10.7; F_1 highest dose, 9.3). These effects were attributed to the strong general systemic parental toxicity at the highest dose. There were no adverse effects on reproductive parameters in either generation at 9.7 or 95.6 mg/kg bw per day.

Pup-weight gain was significantly retarded at the highest dose in both generations from either postnatal day 4 or postnatal day 7 (Table 26). However, there was no effect of treatment on pup viability (stillborn pups, postimplantation losses, pup deaths in the neonatal period or later in the lactation period were all similar to controls). There was no effect on pup growth or viability at lower doses. Preputial separation was significantly delayed by an average of 2.5 days in F_1 males at the highest dose. While time to vaginal opening was slightly shorter in females at the highest dose, this difference was not statistically significant and the value was within the range for historical controls. The slight delay in preputial separation is attributed to the retarded growth recorded in these pups during lactation.

Because of the delay in preputial separation time in the F_1 generation, anogenital distance at birth was measured in rats in the F_2 generation. The anogenital distance of F_2 pups at birth was slightly but significantly greater in males and females at 726 mg/kg bw per day and in males at 95.6 mg/kg bw per day. This finding, which for all males was within the range for historical controls, is attributed to the slightly higher birth weight of these pups and the 0.4 days increase in duration of gestation experienced by F_1 dams.

Table 26(a). Summary of litter data (including developmental milestones) in a multigeneration study in rats given prothioconazole by gavage: F_0 generation adults and F_1 pups

Parameter	Dose (mg/kg bw per day)			
	0	9.7	95.6	726
No. of litters	24	30	29	28
Total No. of pups born	258	334	331	281
Total No. of pups missing	2	2	2	8
Total No. of pups dying	3	1	5	4
Total No. of pups cannibalized	0	0	0	0
Mean No. of pups in litter	10.8	11.1	11.4	10.0
Mean weight of M + F pups (g):				
Day 0	5.9	5.7	5.8	5.9
Day 4 (before cull)	9.5	9.4	9.2	8.6*
Day 7	14.6	15.1	14.4	12.5**
Day 14	29.2	29.2	28.2	24.2**
Day 21	44.6	45.5	42.8	38.2**
Sex ratio at birth (% males)	48.7	44.8	47.6	53.1
No. of stillborn pups	2	3	11	1
Mean No.of viable pups at:				
Birth	11	11	11	10
Day 4 (before cull)	11	11	11	10
Day 4 (after cull)	8	8	8	8
Day 21	8	8	8	8
Live birth index[a]	99.1	99.1	97.1	99.7
Viability index[b]	99.4	99.8	95.6	96.1
Lactation index[c]	98.4	98.3	99.2	98.4
Birth index[d]	90.7	95.3	93.4	91.9
Preputial separation (days) (e Range for historical controls, 41.3–45.9 days)	44.0	44.1	45.1	46.5**
Vaginal opening (days) (e Range for historical controls, 33.1–38.2 days)	35.1	34.5	35.7	33.8

From Young (2001a)

* $p < 0.05$; ** $p < 0.01$ (Dunnett's test)

F, female; M, male.

[a] Live birth index = No. of live-born pups per litter/total No. of pups per litter × 100

[b] Viability index = No. of live pups on day 4 before cull per litter/No. of live pups born per litter × 100

[c] Lactation index = No.of live pups on day 21 per litter/No. of live pups on day 4 after cull per litter × 100

[d] Birth index = total No. of pups born per litter/total No. of implantation sites per dam × 100

[e] Range for historical controls from seven studies in Wistar rats performed in 1998–2001.

Table 26(b). Summary of litter data (including developmental milestones) in a multigeneration study in rats given prothioconazole by gavage: F_1 generation adults and F_2 pups

Parameter	Dose (mg/kg bw per day):			
	0	9.7	95.6	726
No. of litters	27	26	28	25
Total No. of pups born	276	274	271	212
Total No. of pups missing	2	4	8	4
Total No.of pups dying	3	0	4	0
Total No. of pups cannibalized	2	0	0	1
Mean No. of pups in litter	10.2	10.5	9.7	8.2
Mean weight of M + F pups (g):				
Day 0	5.8	5.8	6.0	6.1
Day 4 (before cull)	9.4	9.5	9.7	9.5
Day 7	14.5	14.8	14.8	13.7
Day 14	28.9	29.8	29.0	25.5**
Day 21	43.4	44.7	44.3	40.0*
Sex ratio at birth (% males)	48.6	56.2	53.2	48.4
No.of stillborn pups	2	0	2	3
Mean No. of viable pups at:				
Birth	10	10	10	8
Day 4 (before cull)	10	10	9	8
Day 4 (after cull)	8	8	8	7
Day 21	8	8	8	7
Live birth index[a]	99.4	99.3	99.2	96.2
Viability index[b]	98.3	98.5	96.3	97.6
Lactation index[c]	99.0	99.6	99.6	100
Birth index[d]	93.4	94.2	87.2	87.5
Anogenital distance (mm) at birth:				
Males (Historical controls, 3.76–4.39 mm)[e]	4.20	4.20	4.33*	4.36*
Females (Historical controls, 1.91–2.24 mm)[e]	2.24	2.26	2.27	2.35*

From Young (2001a)

* $p < 0.05$; ** $p < 0.01$ (Dunnett's test)

F, female; M, male.

[a] Live birth index = No. of live-born pups per litter/total No. of pups per litter × 100

[b] Viability index = No. of live pups on day 4 before cull per litter/No. of live pups born per litter × 100

[c] Lactation index = No.of live pups on day 21 per litter/No. of live pups on day 4 after cull per litter × 100

[d] Birth index = Total No. of pups born per litter/total No. of implantation sites per dam × 100

[e] Range for historical controls from 10 studies in Wistar rats performed in 1998–2001.

Of the testicular sperm parameters examined, the only one showing a statistically significant change was total sperm count in the F_1 generation. The values were: control group, 99.6; lowest dose, 86.4; intermediate dose, 79.6 and highest dose, 85.2 per 20 microscope fields. The range for relevant historical controls for the strain and laboratory was 96.7–121.3. In evaluating the observation, is the Meeting noted that only the count for the control group was in the historical range, but that there was a lack of a dose–response relationship. Also, the epididymal sperm count was not reduced, there was an absence of histopathological findings in the testes and there was an absence of treatment-related effects recorded on sperm morphology or sperm motility in the F_1 generation. These parameters were not evaluated in the parental (F_0) generation, apparently because the samples were poorly stored.

There were no notable findings on gross necropsy in pups. In male and female pups (F_1 and F_2), reduced spleen weights were recorded at 726 mg/kg bw per day. There were no notable histopathological changes observed in pups at any dose.

In response to the abnormalities recorded in estrous cycles at 726 mg/kg bw per day, which are consdered to be related to the strong overall maternal toxicity at this dose, a quantitative evaluation of the primordial ovarian follicles was conducted on 10 randomly selected females per group (controls and highest dose for the parental (F_0) generation, all groups for the F_1 generation. The number of pre-antral follicles, and to a lesser extent the number of antral follicles, was increased at all doses in the F_1 generation but without any dose–response relationship (Table 27). In contrast, the number of pre-antral follicles was lower than in the controls and antral follicles were not affected in the F_0 generation. The number of corpora lutea was significantly lower in the F_0 generation at 726 mg/kg bw per day, but was not affected in the F_1 generation (counts were variable and did not show any relationship to dose). On the basis of the inconsistency of these results and the overall unaffected reproductive outcome, the Meeting considered that these changes did not represent a treatment-related effect.

The NOAEL for parental toxicity was 9.7 mg/kg bw per day on the basis of clinical signs in F_0 and F_1 generations, body-weight gain reductions in the pre-mating phases of the F_0 and F_1 generations and organ-weight changes at 95.6 mg/kg bw per day. The NOAEL for reproductive effects was 95.6 mg/kg bw per day on the basis of disruption to the estrous cycle, slight reductions in implantation sites and litter size, increased time to insemination and minimally-increased duration of gestation at 726 mg/kg bw per day. These mild effects were recorded at a dose that also gave rise to marked general systemic parental toxicity; hence, a specific effect on the reproductive system is not indicated. The NOAEL for effects upon the offspring was 95.6 mg/kg bw per day on the basis of reduced pup-weight gain, reduced pup spleen weights and delayed preputial separation at 726 mg/kg bw per day. These effects were recorded at a dose that gave rise to marked general parental toxicity;

Table 27. Summary of quantitative evaluation of ovaries (F_0 and F_1 generations) in a multigeneration study in rats given prothioconazole by gavage

Generation	Ovary parameter	Dose (mg/kg bw per day)			
		0	9.7	95.6	726
F_0	Pre-antral follicles	126.8	—	—	99.4
	Antral follicles	95.1	—	—	100.1
	Corpora lutea	62.4	—	—	36.1*
F_1	Pre-antral follicles	55.2	76.2	70.5	71.8*
	Antral follicles	42.5	52.9	54.9	54.0
	Corpora lutea	28.5	22.2	33.6	22.6

From Young (2001a)

* $p < 0.05$ (Dunnett's test)

—, not determined

hence a specific effect as developmental toxicity was not indicated. The Meeting concluded that prothioconazole is not a selective reproductive toxin (Young, 2001a).

(b) Developmental toxicity

Rats

A non-GLP range-finding study was performed to determine suitable doses for studies of developmental toxicity in rats. Only a summary report of that study was prepared. Groups of mated Wistar rats were given prothioconazole (purity not specified) at a dose of 100 mg/kg bw per day by gavage in 0.5% aqueous Tylose from day 6 to day 15 of gestation, or at 300 or 1000 mg/kg bw per day by gavage in 0.5% aqueous Tylose on day 6 to day 19 post coitum. On day 20 of gestation, the females were killed and assessed by gross pathology. The dams were examined for general tolerance of prothioconazole and intrauterine development (implantations and resorptions). Body weights and food and water consumption were recorded. The fetuses were examined for external abnormalities and for the presence of supernumerary ribs.

There were no treatment-related deaths or clinical signs of an adverse effect of treatment at any dose, but increased water consumption occurred at 1000 mg/kg bw per day. Slightly reduced body-weight gain early in the treatment period occurred at doses of 300 and 1000 mg/kg bw per day, but not at 100 mg/kg bw per day. Food consumption of the dams was unaffected by treatment at all doses. Although the incidence of resorptions was elevated at 300 mg/kg bw per day, a similar effect was not evident at 1000 mg/kg bw per day. There were no treatment-related external abnormalities detected at doses up to and including 1000 mg/kg bw per day. Increased incidences of supernumerary ribs occurred at 300 mg/kg bw per day (24% of fetuses with 14th or cervical ribs) and 1000 mg/kg bw per day (12% of fetuses with 14th ribs). No supernumerary ribs occurred at 100mg/kg bw per day. There appeared to be no skeletal abnormalities, although a detailed examination of the specimens was not conducted (Kolb, 1995).

In a study of developmental toxicity, groups of 26 time-mated, female Hsd.Cpb:WU Wistar rats were given prothioconazole (purity, 99.5–99.8%) at a dose of 0, 80, 500 or 1000 mg/kg bw per day by gavage in 0.5% aqueous carboxymethylcellulose on days 6 to 19 post coitum. The day of confirmation of mating (when spermatozoa were detected) was designated day 0 of gestation. On day 20 of gestation, the females were killed and assessed for gross pathology. Liver weights were recorded and histopathological examinations were performed on liver, thyroid, adrenal glands and tissues with gross findings from all dams. Samples of blood and liver were also taken from 18–22 females per group for determination of AST, ALT, ALP, glutamate dehydrogenase (GLDH), cholesterol, triglycerides, TSH, T3 and T4 in blood, and triglycerides in liver. 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. The study was compliant with or exceeded the requirements of the contemporary OECD guideline 414 (1981). Dosing was extended from day 15 to day 19 in anticipation of changes to US EPA guidelines for this type of study (which brought the dosing pattern in this study in line with the 2001 OECD guideline 414).

The accuracy, stability and homogeneity of the dosing solutions were confirmed by analysis.

There were no deaths. The only clinical observation was increased urination at 500 and 1000 mg/kg bw per day. Food consumption was significantly reduced at the highest dose during days 6–11 of gestation (Table 28). The measured water consumption was drastically increased at 500 and 1000 mg/kg bw per day throughout the treatment period. Significantly reduced body-weight gains during gestation were recorded at 500 and 1000 mg/kg bw per day, with rats at 1000 mg/kg bw per day showing transient body-weight loss over days 6–8 of gestation.

Consistent with the results obtained in other multiple-dose studies with rats, kidney dysfunction and disturbed systemic water homoeostasis (dehydration) were the primary maternal toxicological effects of prothioconazole and were considered to be life-threatening in their severity.

The results of blood-chemistry analyses are presented in Table 29. ALT, ALP activities and cholesterol concentrations were slightly but statistically significantly increased at 1000 mg/kg bw per day, but AST activity was lower than in the controls. Cholesterol concentration was also raised at 500 mg/kg bw per day, with no dose–response relationship. There were no effects on GLDH or on triglycerides in either the blood or the liver. Of the thyroid hormones, concentrations of T4 were significantly lower at 500 and 1000 mg/kg bw per day (in a dose-dependent manner), concentrations of T3 were lower (not statistically significant) and concentrations of TSH were higher in rats receiving prothioconazole (not statistically significant, no dose–response relationship).

Relative liver weights were significantly increased at 1000 mg/kg bw per day by 6% above values for the controls. The only notable finding on gross examination was a single rat at 1000 mg/kg bw per day with material deposited in the ureter and urinary bladder along with multiple white areas in the kidneys that was confirmed microscopically as urolithiasis with urothelial hyperplasia and hydronephrosis with pyelonephritis and transitional-cell hyperplasia. Microscopic examination of the liver, thyroid, adrenals and apparent gross abnormalities did not reveal any other notable findings.

The incidences of pregnancy and the mean numbers of corpora lutea and implantations were comparable between all test and control groups. Preimplantation and postimplantation losses, live litter size, placental weight and fetal sex ratios were unaffected by treatment at any dose. The incidences of engorged placentas were increased, relative to values for the controls, at all doses. However the fetal incidences at 80 and 500 mg/kg bw per day were within the range for historical controls

Table 28. Food and water consumption and body-weight gains in a study of developmental toxicity in rats given prothioconazole by gavage

Parameter	Group mean values for maternal rats			
	Dose (mg/kg bw per day)			
	0	80	500	1000
Food consumption (g/day):				
Days 0–6	20.1	20.5	19.8	20.4
Days 6–11	18.6	19.3	17.8	15.4**
Days 11–16	21.6	21.7	21.3	20.7
Days 16–20	22.7	22.5	22.8	23.1
Water consumption (g/day):				
Days 0–6	26.5	26.8	26.7	29.1
Days 6–11	27.0	27.3	33.2*	47.3**
Days 11–16	30.5	31.6	38.6**	49.9**
Days 16–20	33.3	34.2	43.6**	52.8**
Body-weight gain (g):				
Days 6–19	72.8	71.2	70.4	64.8
Days 0–20	102.0	101.7	99.4	92.6
Days 0–20 (corrected for uterus weight)	40.0	39.4	31.5*	27.6**

From Stahl (1997)

* $p < 0.05$; ** $p < 0.01$ (Dunnett's test)

Table 29. Summary of notable clinical chemistry results in a study of developmental toxicity in rats given prothioconazole by gavage

Parameter	Group mean values for maternal rats			
	Dose (mg/kg bw per day)			
	0	80	500	1000
AST (U/l)	37.5	38.9	32.8**	34.0*
ALT (U/l)	40.7	43.0	42.7	47.6**
ALP (U/l)	101	99	105	134**
Cholesterol (mmol/l)	2.25	2.16	2.67**	2.61**
T4 (nmol/l)	28	26	22*	20**
T3 (nmol/l)	1.58	1.51	1.48	1.42
TSH (ng/ml)	3.17	3.43	3.71	3.53

From Stahl (1997)

ALP, alkaline phosphatise; ALT, alanine aminotransferase; AST, aspartate aminotransferase; T3, triiodothyronine; T4, thyroxin; TSH, thyroid-stimulating hormone.

* $p < 0.05$; ** $p < 0.01$ (adjusted Welsh test or Kruskal-Wallis test followed by adjusted U test)

and there was no corresponding effect on placental weight in any group. An effect of treatment was considered to have occurred only at 1000 mg/kg bw per day, since this incidence was outside the range for historical controls. Fetal weights of males and females at 1000 mg/kg bw per day were significantly reduced by about 4–5%.

There was an apparent increase in the incidence (both fetal and litter-based) of microphthalmia in groups receiving prothioconazole compared with the control group. Although the incidence at 80 and 500 mg/kg bw per day was not dose-related, it is notable that the incidence was zero in 290 fetuses of the control group (Table 30).

The study was conducted in 1996. Data on historical controls for Hsd Cpb:WU Wistar rats during 1990–2002 included 21 other studies with zero incidence and 27 studies in which the fetal incidences ranged from 0.3% to 1.95% and the litter incidences ranged from 3.8% to 20.0%. The incidence of microphthalmia in the group at the highest dose was outside the range for historical controls and there were also two fetuses with bilateral microphthalmia in the group at the highest dose (considered more likely to indicate an effect of treatment).

Microphthalmia often showed a high variability between historical control groups within a given study conducted in the same rat strain and laboratory as the present study (Table 31). Therefore, it is difficult to have confidence in the data generated in this particular study. The incidences of microphthalmia at 80 and 500 mg/kg bw per day were assessed as indicators for the high variability of this (in the present rat strain) common spontaneous malformation, but not as a treatment-related effect. Only the increase at 1000 mg/kg bw per day was found to be related to treatment.

The only notable visceral finding other than microphthalmia was dilatation of the renal pelvis, which was recorded at 7.2%, 2.9%, 7.8% and 17.0% in fetuses in the groups at 0, 80, 500 and 1000 mg/kg bw per day, respectively (Table 32). This finding was considered to be secondary to retarded fetal development at this dose, as indicated by decreased fetal weight and increased incidences of incomplete ossification (distal and proximal phalanges, caudal vertebral bodies and sixth sternebral bone).

Table 30. Incidence of external malformations and microphthalmia in a study of developmental toxicity in rats given prothioconazole by gavage

Parameter	Fetal (litter) incidences (%)			
	Dose (mg/kg bw per day)			
	0	80	500	1000
External examination				
No. of litters evaluated	26	26	22	24
No. of fetuses evaluated	290	292	270	282
Microphthalmia	0 (0.0)	0.3 (3.8)	0 (0.0)	0.7 (4.2)
Eye rudiment flat	0 (0.0)	2.1 (11.5)	0.4 (4.5)	2.1 (20.8)
Visceral examination				
No. of litters evaluated	26	25	22	24
No. of fetuses evaluated	138	137	128	135
Microphthalmia (Wilson's technique)	0 (0.0)	4.4* (16.0)	2.3 (13.6)	8.1**(33.3)**
Microphthalmia (all fetuses)[a]	0 (0.0)	2.4 (15.4)	1.1 (13.6)	4.6 (33.3)

From Stahl (1997)

* $p < 0.05$; ** $p < 0.01$.

[a] All fetuses that showed "microphthalmia" or "eye rudiment flat" at external examination were thereafter assigned to the subgroup for visceral examination. Any case of microphthalmia that might be missed at external examination for a fetus that was thereafter assigned to skeletal evaluation would be detected at skeletal evaluation as "eyehole reduced in size". It was considered appropriate to combine external, visceral and skeletal incidences of microphthalmia to derive an 'all fetuses' value (related to all (viscerally and skeletally) examined fetuses) for the purpose of comparison to the data for historical controls.

Table 31. Examples of inter-group variability in the incidence of microphthalmia in Hsd Cpb:WU Wistar rats

Year	Study number	Litter incidence of microphthalmia (%)			
		Control	Lowest dose	Intermediate dose	Highest dose
1995	T2055246	17.9	6.5	6.3	17.2
1996	Prothioconazole	0	15.4	13.6	33.3
1997	T0060860	20.0	0	4.2	27.8
2002	T6071558	20.0	12.5	4.8	0

From Stahl (1997)

There were statistically significantly increased, dose-related incidences of rudimentary (punctiform and comma-shaped) supernumerary 14th lumbar ribs in all treated groups compared with controls. No fully formed 14th ribs were observed at any dose.. Rudimentary supernumerary ribs are a common spontaneous variation in untreated rats. Between 1992 and 1999, the percentages of fetuses with this variation were between 0.7% (this study) and 9.4% and the percentages of litters with the same variation were between 3.8% (this study) and 40% in a database of 24 studies, which included one without the variation. The fetal and litter incidences of this finding at 80 and at 500 mg/kg bw per day were within or at the upper bound of the range for historical controls, while the incidence at 1000 mg/kg bw per day was outside the range for contemporary historical controls and was considered to be a treatment-related effect. However, there were clear time trends within the

historical-control database and consideration of a timeframe of 1996 ± 2 years (the date of this study) provides a different picture, in which the upper bounds of the range for historical controls were 5.6% of fetuses and 21.7% of litters with rudimentary supernumerary 14th ribs. On this basis, all groups receiving prothioconazole had this variation at incidences above the upper bound of the range for historical controls. There were no notable increases in any other findings and the overall incidences of abnormal fetuses and litters in all treated groups were not significantly different from those in the control group.

The NOAEL for dams was 80 mg/kg bw per day on the basis of reduced body-weight gains, and increased water consumption and urination at 500 mg/kg bw per day.

A NOAEL for developmental toxicity could not be identified owing to a statistically significant and dose-related increase in the incidence of rudimentary supernumerary 14th ribs at all doses, including 80 mg/kg bw per day, the lowest dose tested. At higher doses there were increased incidences of engorged placentas, retarded fetal development (reduced fetal weights, delayed ossification, renal pelvis dilatation and increased incidences of microphthalmia and supernumerary ribs at 1000 mg/kg bw per day (Stahl, 1997).

Because of concerns with the previously described study of developmental toxicity (Stahl, 1997), a new study was conducted in order to investigate the specificity of microphthalmia formation. A different substrain of rat was selected for which the available database of historical controls revealed a background incidence of microphthalmia of virtually zero. The strain was nevertheless sensitive to a direct, specific oculo-teratogenic effect; at a dose of 15 mg/kg bw per day, the positive control *all trans*-retinoic acid caused increased litter incidences of anophthalmia (41.7%), microphthalmia (16.7%) and small lens (8.3%). In order to establish an objective measure for ocular size, rather than the more subjective standard guideline observation according to the Wilson freehand-slicing technique, fresh fetal eyes were extracted, weighed and morphometrically investigated.

In this second study of developmental toxicity, groups of 25 time-mated, female Crl:WI(HAN) Wistar rats were given prothioconazole (purity, 98.7%) at a dose of 0, 20, 80 or 750 mg/kg bw per day by gavage in 0.5% aqueous carboxymethylcellulose on days 6 to 19 post coitum. The day of confirmation of mating (when spermatozoa were detected) was designated day 0 of gestation. Doses were selected to reflect those of the original study, but extending the range at the lower end and reducing the highest dose from 1000 mg/kg to 750 mg/kg on the basis of the higher sensitivity of this rat strain. In a pilot dose range-finding study of maternal toxicity, there was 25% mortality owing to dehydration at 1000 mg/kg bw per day.

This GLP-compliant study also complied with OECD guideline 414 (2001), but with the following modifications: no visceral investigations were conducted, since the heads of all fetuses were used for special ocular investigations and the torsos of all fetuses were used for a complete skeletal investigation with special emphasis on rib alterations. These modifications provided increased power to investigate the effects identified previously as being of primary concern.

According to the study objectives, some aspects of the study design deviated from the standard guideline programme. Maternal evaluations included organ-weight determination, clinical chemistry and histopathology to investigate possible effects on kidneys and liver, in addition to the minimum requirements. Fetal visceral examinations were not conducted since all fetuses were decapitated and the torso was evaluated for general skeletal and cartilage development with emphasis on the occurrence of supernumerary rudimentary (punctiform and comma-shaped) and extra ribs. All fetal heads were skinned, and fetal brains and eyes (distinguishing right from left) were extracted and weighed. The eyes were photographed so that both the horizontal and vertical diameters of the entire eye and of the cornea (including the area of the cornea), as well as the longitudinal length of the distance from the optic nerve remnant to the very front of the cornea, could be measured (see Figure 3 for an example).

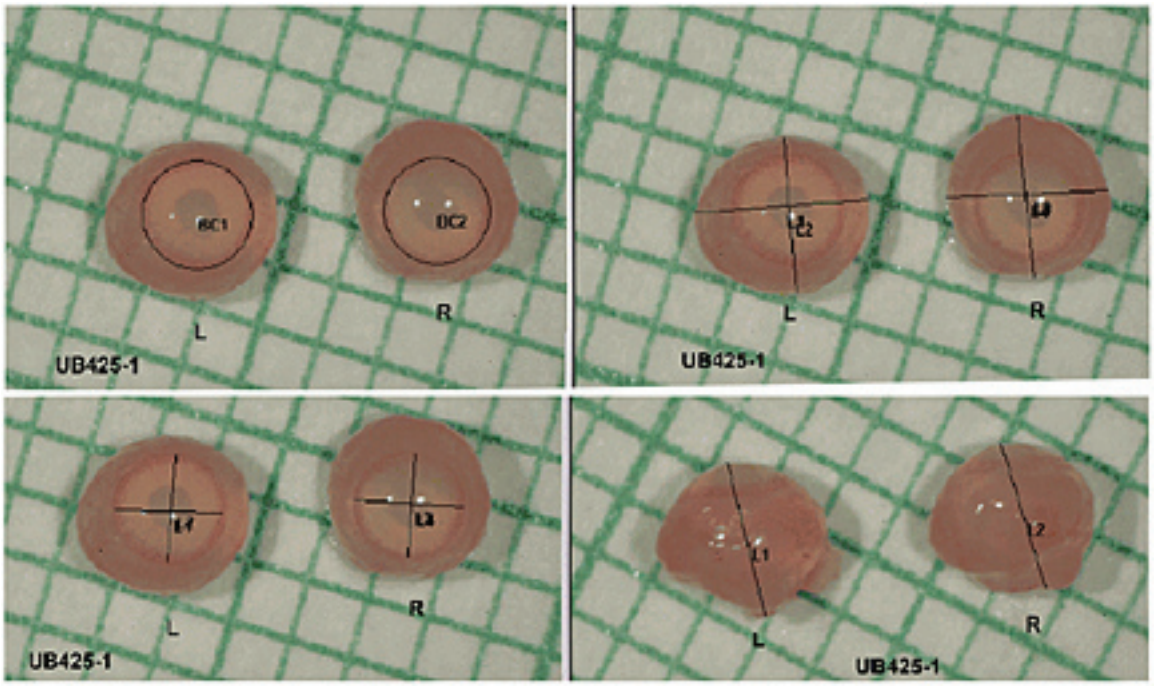


Figure 3. Illustration of morphometric measurements in freshly extracted fetal rat eyes (scale: one square = 1 mm²)

At 750 mg/kg bw per day, there was a 13% decrease in net body-weight gain (i.e. excluding gravid uterus) during gestation and a 46% reduction in overall body-weight gain during days 6–12 of gestation. Also, there was a 60% increase in water consumption and an 18% decrease in food consumption. There were changes in clinical chemical indicators of functional impairments at this dose in the kidneys (blood concentration of urea nitrogen increased significantly from 16 to 19 mg/dl) and liver (blood concentration of cholesterol significantly increased from 83 to 98 mg/dl and ALP activity increased from 65 to 104 U/l, while AST activity decreased significantly from 67 to 56 U/l). These results correlated well with the established toxicological profile of prothioconazole.

There were neither treatment-related reproductive effects nor significant differences in the litter size, the median percentage of male fetuses, fetal weights or placental weights in any group tested. No treatment-related effects were observed on external or skeletal malformations or on external variations.

The external examinations before and after skinning did not reveal any fetus with microphthalmia in any treated group. No compound-related effects were observed on the individual or mean eye weights, eye-to-fetal weight ratios, or on eye measurements (Figure 4). Thus, there was no evidence that prothioconazole caused microphthalmia in any treated group.

Skeletal evaluation revealed a possible treatment-related increase in the fetal incidence of supernumerary rudimentary (comma-shaped) ribs at 750 mg/kg bw per day, while the litter incidence was not significantly affected (Table 33). A treatment-related effect on punctiform or fully-formed supernumerary ribs was not discernible. The fetal incidence of comma-shaped rudimentary ribs (21.2%) was marginally higher than the upper bound (18%) of the range for historical controls for the same laboratory and rat strain and the incidence of punctiform ribs (33.6%) was well below the upper bound (52%) of the range for historical controls. The Meeting noted, however, that the litter incidence of punctiform ribs in the control group was 95.2%, rendering the possibility of demonstrating any increase extremely difficult. There were no alterations in supernumerary ribs at 80 mg/kg bw

per day. The marginal increase in the incidence of rudimentary (comma-shaped) supernumerary ribs at 750 mg/kg bw per day was considered to be an enhancement of this common variation secondary to the marked maternal toxicity observed at this dose.

The NOAEL for maternal toxicity was 80 mg/kg bw per day on the basis of decreased net body-weight gain, markedly increased water consumption, decreased food consumption and clinical chemical indications for functional impairments of kidneys and liver at 750 mg/kg bw per day. The NOAEL for developmental toxicity was 80 mg/kg bw per day on the basis of a marginal increase in the incidence of fetal supernumerary rudimentary (comma-shaped) ribs at 750 mg/kg bw per day (Young, 2004).

In a study of developmental toxicity, groups of 29–30 mated, female Wistar rats received prothioconazole by topical application on days 6 to 19 post coitum. The actual numbers of pregnant rats per group were 17–23. The test material was either: technical prothioconazole as a dry powder (purity, 98.1–98.8%) at a dose of 1000 mg/kg bw per day, moistened with water; or a formulation (EC250) containing 25% prothioconazole at a dose of 1000 mg/kg bw per day (equivalent to prothioconazole at 250 mg/kg bw per day); or a 1 : 3 aqueous dilution of the above formulation (EC250) at a dose of 1000 mg/kg bw per day (equivalent to prothioconazole at 62.5 mg /kg bw per day; or deionized water only (vehicle control).

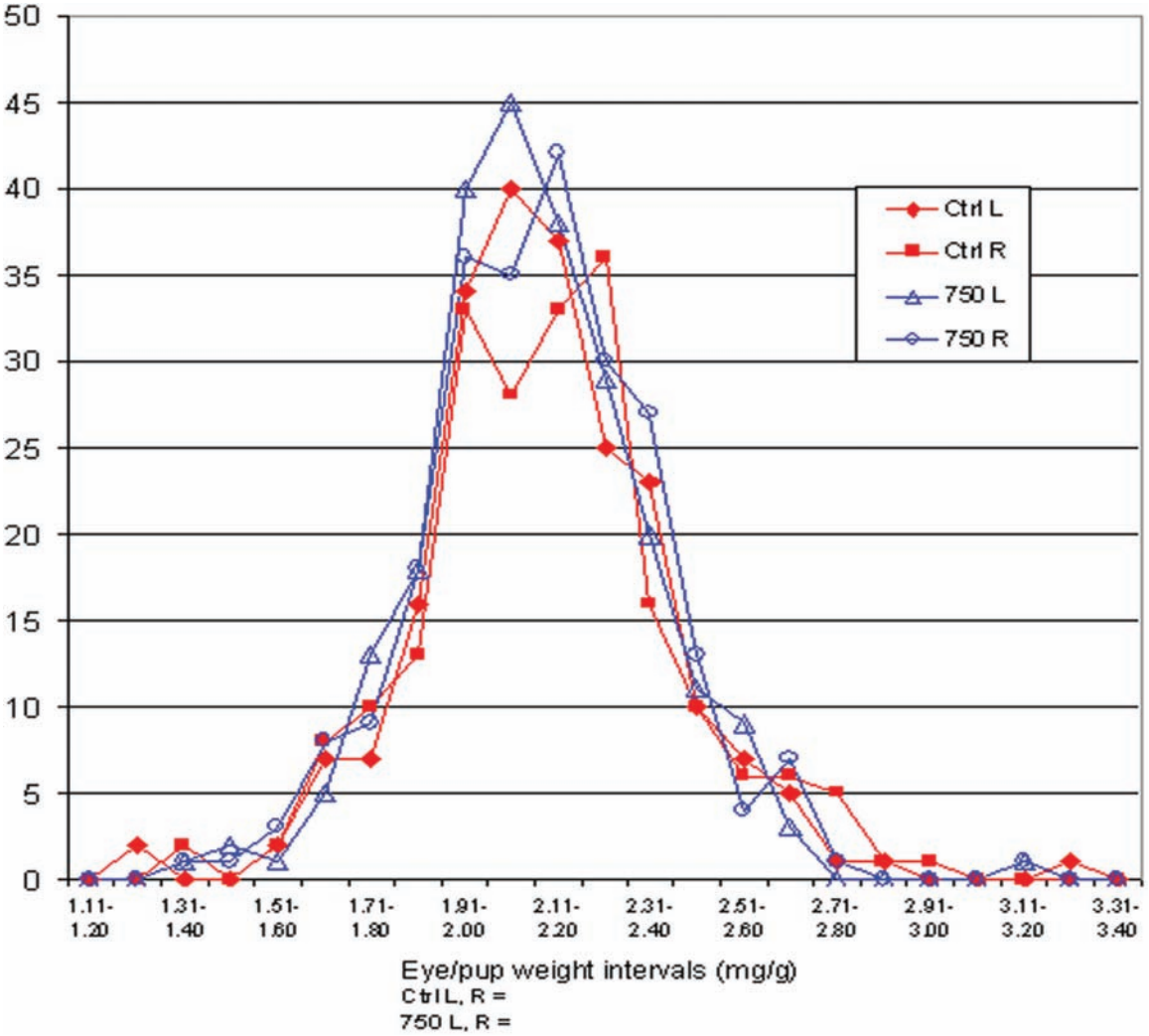
The test material was applied for 6 h per day, on non-occlusive dressings, to an area of clipped skin equivalent to 10% of the body surface area. The treatment sites were wiped with water after removal of the dressings. In-life observations made for the dams were clinical signs, body weights and food consumption. On day 20 of gestation, the females were killed and assessed for gross pathology. The gravid uterus was weighed, the fetuses were removed and the placentas were weighed and examined. The position of fetuses in the uterine horns, the numbers of implantation sites and corpora lutea, fetal weights and sexes were recorded. Implantation sites were characterized. Fetuses were examined externally for malformations. Approximately one half of the fetuses from each litter were examined fresh for visceral abnormalities. Cranial examination was performed by razor-sectioning of Bouins-fixed specimens. The carcasses of the remaining fetuses were stained for the examination

Table 33. Mean incidences (%) of supernumerary 14th ribs in a study of developmental toxicity in rats given prothioconazole by gavage

Characteristic of supernumerary rib	Mean incidence (%)			
	Dose (mg/kg bw per day)			
	0	20	80	750
Rudimentary ribs (punctiform):				
Fetal incidence (%)	23.5	18.2	27.6	33.6
Litter incidence (%)	95.2	77.8	88.9	95.7
Rudimentary (comma-shaped):				
Fetal incidence (%)	11.8	7.4	12.4	21.2*
Litter incidence (%)	52.4	66.7	38.9	69.6
Extra (full-size):				
Fetal incidence (%)	6.3	3.4	1.2*	8.3
Litter incidence (%)	33.3	16.7	11.1	39.1

From Young (2004)

* $p \leq 0.05$; ** $p \leq 0.01$



From Young (2004)

Figure 4. Distribution of fetal eye weights (relative to body weight) in a study of developmental toxicity in rats given prothioconazole by gavage

of the ossified and cartilaginous skeleton for skeletal abnormalities and variations. Skeletal variations were defined as alterations in development commonly observed in the strain employed.

The study complied with or exceeded the requirements of the contemporary OECD guideline 414 (1981). Dosing was extended from day 15 to day 19 to comply with the appropriate US EPA guideline (which brought the dosing pattern in this study in line with the 2001 OECD guideline 414). The dose of 1000 mg/kg bw per day is a limit dose for this type of study.

The accuracy, stability and homogeneity of the diluted EC250 dosing solution were confirmed by analysis. The other test materials were used undiluted as supplied.

There were no deaths. There were signs of skin irritation noted in rats treated with the undiluted EC250 formulation, consisting of erythema and oedema in one rat, eschar formation in six rats and scaling and sloughing of the application site in twenty rats. Red vaginal discharge was observed sporadically in all treated groups except the control group, in both pregnant and non-pregnant rats, on days 14–17 of gestation. The incidences were 4 out of 29, 6 out of 30 and 23 out of 29 in the groups treated with diluted EC250 formulation, undiluted EC250 formulation and prothioconazole technical, respectively. A supplementary study was performed to investigate the relationship of this finding to

treatment with prothioconazole. From the results of the supplementary study (see below), the Meeting concluded that the red vaginal discharge was not related to treatment with prothioconazole.

There were no effects of treatment on body weights, food consumption, gross findings at autopsy, uterine weight, the number of corpora lutea, implantation sites, or pre- or postimplantation losses in any treatment group. Increased mean male fetal weight was recorded in the group receiving the diluted EC250 formulation, but no similar effect was recorded in the group receiving the undiluted formulation and this finding was not considered to be toxicologically significant. All other litter parameters were similar in the treated and the control groups.

The overall litter and fetal incidences of abnormalities were comparable to those in the controls in all treated groups and the nature of the abnormalities recorded did not indicate any particular effect of treatment. The pattern of malformations, visceral abnormalities and variations and skeletal abnormalities between the control and treated groups did not indicate any effect of treatment. Incidences of skeletal variations were lower in the treated groups compared with the controls (not considered to be toxicologically significant).

Skin application of the undiluted EC250 formulation was associated with skin irritation. There were no systemic toxic effects identified in any group, and no effects on developing offspring. The NOAEL for maternal and developmental toxicity afer dermal application was 1000 mg/kg bw per day for prothioconazole technical material, 1000 mg/kg bw per day for the EC250 formulation (equivalent to prothioconazole at 250 mg/kg bw per day) and 1000 mg/kg bw per day for the diluted EC250 formulation (equivalent to prothioconazole at 62.5 mg/kg bw per day).

A supplementary study was performed to study the possible effect of prothioconazole administration on vaginal discharge in rats. Groups of 5 or 10 mated female Wistar rats were treated as follows. Ten females received prothioconazole at a dose of 1000 mg/kg bw per day by dermal application on days 6–19 of gestation (replicating the main study). Ten females received deionized water applied directly to the vaginal area (on the surface of the vaginal opening) on days 6–19 gestation. Ten females received a small amount of prothioconazole technical (dry material plus a small amount of deionized water) applied directly to the vaginal area on days 6–19 of gestation, the mean amount of prothioconazole plus water applied being 33 mg (range, 13.5–58.1 mg). Five females received coloured dye at a dose of 1000 mg/kg bw per day on days 6–8 gestation, to determine whether there was the potential for dry material to migrate from the application site. These rats were killed on day 8 after it had been verified that dye material was observed in areas outside the application site.

In all other respects, the study replicated the conditions of the main study of dermal application study described above, i.e. rats wore gauze patches (dry gauze in the case of vaginally-treated rats), collars etc.). For five rats in the control group receiving water only (direct vaginal application) and the group receiving prothioconazole at a dose of 1000 mg/kg bw per day by dermal application, serum and vaginal-wipe samples were taken and analysed for the presence of prothioconazole. A gross autopsy and microscopic examination of the uterus, cervix and vagina was also performed.

In rats receiving prothioconazole, red vaginal discharge was observed in 8 out of 10 rats between days 13 and 18. In rats receiving water only, red vaginal discharge was observed in 6 out of 10 rats, between days 13 and 18. In rats receiving prothioconazole by vaginal application, red vaginal discharge was observed in 8 out of 10 rats between days 12 and 18. Prothioconazole was detected in the serum of rats receiving prothioconazole dermally but not in rats receiving water only, thereby showing that there had not been cross-contamination by prothioconazole. Prothioconazole was also detected in the vaginal wipes of rats receiving prothioconazole dermally, but not of rats receiving water only. Possibly, the test material was present in the vaginal area either by transfer from the application site during grooming (despite the presence of gauze and collars during the application period), or via urinary excretion of the test compound. There were no notable effects on body weights

or food consumption and no notable findings at gross necropsy or after microscopic examination of the uterus, cervix or vagina.

The fact that red vaginal discharge was recorded in rats in the control group that had not been exposed to prothioconazole indicated that this clinical sign was not related to treatment with prothioconazole. The fact that prothioconazole was not found in either the serum or the vaginal wipes of rats in the control group showed that there was no cross-contamination between groups and that the presence of prothioconazole in the vagina was not required for this sign to be observed.

The Meeting concluded that the red vaginal discharge recorded in the study of developmental toxicity with prothioconazole administered dermally was not an effect of treatment. Clearly, this discharge was a normal physiological occurrence, and the frequency with which it was recorded may be related to the quantity of the discharge and the ability of the dam to effectively groom the vaginal region. This discharge was noted in these particular studies because the rats were wearing collars, which may have inhibited normal grooming during the exposure period in some rats (depending on the position of the collar and the flexibility of each individual rat) (Young, 2001b).

Rabbits

A non-GLP range-finding study was performed to determine suitable doses for studies of developmental toxicity in rabbits. Groups of three or five mated Chinchilla rabbits were given prothioconazole (purity, 99.7%) at a dose of 480, 100, 300 and 80 mg/kg bw per day (sequentially in that order) by gavage in 0.5% aqueous carboxymethylcellulose from day 6 to day 27 of gestation. Clinical signs, body weights and food consumption of the dams were recorded. On day 28 of gestation, the females were killed and assessed by gross pathology. Uterine and implantation-site parameters were assessed and fetuses received a restricted examination for abnormalities (including degree of ossification of the cranium, a single cross-section through the brain, and abnormalities of the major blood vessels, heart and kidneys by dissection).

One or two deaths were recorded at each dose between days 23 and 27 of gestation. Dose-dependent body-weight loss and persistently reduced food consumption was recorded in all groups dosed. Total postimplantation loss was recorded in one rabbit at 480 mg/kg bw per day, and fetal weights were reduced at 480 mg/kg bw per day, but not at lower doses. There was no effect on other litter parameters. There were no treatment-related findings on autopsy (although all rabbits except one decedent had dark-red discoloration of the lungs). Nine of the 16 fetuses obtained from dams treated at 480 mg/kg bw per day were runts (small fetuses with body weights of 9.8–17.7 g, compared with a mean body weight in other groups of approximately 30 g). One fetus from the group at 80 mg/kg bw per day showed encephalocele in the region of the large fontanelle. No other internal or external anomalies were identified.

A NOAEL was not identified in this study (< 80 mg/kg bw per day). There were no reproductive effects identified, but reduced fetal growth occurred at 480 mg/kg bw per day (Becker & Biedermann, 1997).

Groups of 24 mated female Chinchilla rabbits were given prothioconazole (purity, 99.7%) at a dose of 0, 10, 30 or 80 mg/kg bw per day by gavage in 0.5% aqueous carboxymethylcellulose from day 6 to day 27 post coitum. An additional group of 24 rabbits receiving a dose of 350 mg/kg bw per day was added to the study owing to the absence of clear maternal toxicity at 80 mg/kg bw per day. A further six and seven mated females were treated at 10 and 80 mg/kg bw per day, respectively, owing to low incidences of pregnancy. In-life observations in dams were clinical signs, body weights and food consumption. Fetuses were delivered by caesarean section on day 28 of gestation, a gross autopsy was performed on the dams and their livers and adrenal glands were weighed.

The gravid uterus was weighed, the fetuses were removed and the placentas were weighed and examined. The position of fetuses in the uterine horns, the numbers of implantation sites and corpora lutea and fetal weights were recorded. Implantation sites were classified as embryonic (early) resorption, fetal (late) resorption, live fetus or dead fetus. Fetuses were externally examined for malformations. The skin and fat pads were removed from the fetuses, the eyes were examined, the cranium was examined for degree of ossification, and the heads of 50% of fetuses from each litter were serially sectioned and the internal structure of the brain examined. The heads of the remaining fetuses were fixed and then serially sectioned. Thoracic and abdominal organs from all fetuses were examined by microdissection, the sex was determined followed by evisceration. The carcasses of all fetuses were double-stained for cartilage and ossified bone and then examined for skeletal abnormalities and variations. The study complied with or exceeded the requirements of the contemporary OECD guideline 414 (1981) with the dosing pattern and fetal examinations complying with OECD guideline 414 (2001).

The accuracy, stability and homogeneity of the dosing solutions were confirmed by analysis.

There was one death (day 25) and three abortions (days 22, 25 or 27) of rabbits at 350 mg/kg bw per day. There were no treatment-related clinical signs of toxicity. Overall food consumption and overall body-weight gain were statistically significantly reduced at 350 mg/kg bw per day by 31% and 47%, respectively. Lower food consumption was most marked over days 6–19, and body-weight loss was recorded on days 6–11. There were no effects on food consumption or body weights at doses of up to 80 mg/kg bw per day. There were no gross findings at autopsy or effects on liver or adrenal weights of rats at any dose.

In the group at 350 mg/kg bw per day, three rabbits aborted and there were total litter resorptions in three other rabbits, resulting in decreased overall litter size in this group, but not in dams that maintained live litters to day 28. Also in this group at the highest dose, postimplantation losses of 29.6% were significantly higher than the value for controls of 10.2%, mean fetal weights were significantly reduced by 10–13% and mean placental weight was 5.8% lower; however, there was no effect on preimplantation loss, the incidence of dead fetuses or the fetal sex ratio. There were no effects on any reproductive parameters or fetal weights at doses up to 80 mg/kg bw per day.

The nature and incidences of external, visceral and skeletal abnormalities did not indicate an effect of treatment at any dose (Table 34). The percentage incidence of fetuses with any abnormality was similar across all treated groups with no dose–response relationship, which does not suggest a treatment-related effect. The incidence of fetuses with abnormal skeletal findings was well within the normal range for controls of this strain of rabbits (range, 0.0–3.6% in studies carried out in 1993–1995). Although the incidence of fetuses with rib fusion or bifurcation or thoracic vertebral defects at 350 mg/kg bw per day was slightly raised relative to values for concurrent controls (three cases vs one case in the controls), the Meeting considered that this was not a treatment-related effect since incidences of up to four cases in comparable numbers of fetuses had occurred in the laboratory according to data on historical controls from studies performed in 1991–1995. The Meeting suggested that 1995–2001 would be more relevant, since the study was reported in 1998.

In comparison with the study of developmental toxicity with prothioconazole administered by oral gavage in rats, it was notable that there was only a single incidence of microphthalmia in this study (in the group receiving the owest dose), and that was in a fetus with multiple malformations affecting the head.

The incidences of skeletal variants are presented in Table 35. At 350 mg/kg bw per day, there were notable differences from values for the controls in the fetal incidences of incomplete and absent ossification of one or more sternebrae and phalanges of the digits, and of unossified 13th rib. In some cases the incidence was lower and in others the incidence was higher than values for the controls, indicating either advanced or retarded ossification in different structures. This did not suggest a clear effect of treatment, although an increased incidence of retarded ossification would not be unexpected given the lower fetal body weights recorded in this group.

Table 34. Group mean incidence of fetal abnormalities in a study of developmental toxicity in rabbits given prothioconazole by gavage

Parameter	Group mean value				
	Dose (mg/kg bw per day)				
	0	10	30	80	350
No. of litters evaluated	20	24	21	23	17
No. of fetuses evaluated (external)	176	210	190	205	152
No. of litters with any external/fresh visceral abnormality (%)	0 (0.0)	3 (12.5)	0 (0.0)	3 (13.0)	1 (5.9)
No. of fetuses with any external/fresh visceral abnormality (%)	0 (0.0)	4 (1.9)	0 (0.0)	2.0)	1 (0.7)
No. of fetuses evaluated (heart + vessels/head)	176 / 87	210 / 103	190 / 98	205 / 103	152 / 76
No. of litters with any visceral abnormality (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. of fetuses with any visceral abnormality (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. of fetuses evaluated (skeletal)	176	210	190	205	152
No. of litters with any skeletal abnormality (%)	2 (10.0)	2 (8.3)	0 (0.0)	3 (13.0)	4 (23.5)
Fetuses with any skeletal abnormality (%)	2 (1.1)	2 (1.0)	0 (0.0)	3 (1.5)	4 (2.6)
Total No. of litters with any abnormality (%)	2 (10.0)	4 (16.7)	0 (0.0)	5 (21.7)	4 (23.5)
Total No. of fetuses with any abnormality (%)	2 (1.1)[a]	5 (2.4)[b]	0 (0.0)	6 (2.9)[c]	4 (2.6)[d]

From Becker & Biedermann (1998)

[a] One fetus with thoracic vertebral defect, one fetus with fused and abnormally ossified sternebrae.

[b] One runt with missing rib and vertebral body, two fetuses with right kidney/ureter agenesis, one fetus with cheilognathopalatoschisis, encephalocele, micro/anophthalmia, prolaps linguae, one fetus with fused and abnormally ossified sternebrae.

[c] Two runts, one with fused and abnormally ossified sternebrae, one fetus with fused ribs, one fetus with abnormally ossified sternebrae, two fetuses with agenesis of left kidney/ureter.

[d] One fetus with enlarged fontanelle, shortened and bent ulna/radius, humerus/tibia/fibula divided and bent, wavy ribs, three fetuses with fused ribs two with some vertebral bodies absent and one with bifurcated ribs.

The NOAEL for maternal toxicity was 80 mg/kg bw per day on the basis of mortality, body-weight loss or reduced body-weight gain and reduced food consumption at 350 mg/kg bw per day. The NOAEL for developmental effects was 80 mg/kg bw per day on the basis of abortion, total litter loss and reduced fetal weights, that were most likely to be due to maternal toxicity, and possibly retarded ossification at 350 mg/kg bw per day. There was no evidence of a teratogenic effect (Becker & Biedermann, 1998)

2.6 Special studies

(a) Delayed neurotoxicity

No data were submitted that would address possible delayed neurotoxicity. Since prothioconazole is not a member of a chemical class associated with delayed neurotoxicity and since there was no evidence of changes in nervous tissues, testing for delayed neurotoxicity was not required.

Groups of 12 male and 12 female Wistar rats were given prothioconazole (purity, 97.6–98.8%) as a single dose at nominal doses of 0, 200, 750 or 2000 mg/kg bw by gavage formulated in aqueous 0.5% methylcellulose and 0.4% Tween 80. The analytically-confirmed doses were 0, 218, 877 and 2240 mg/kg bw. Investigations included clinical signs, body weights (weekly), FOB, motor and

Table 35. Summary of notable skeletal variants found in a study of developmental toxicity in rabbits given prothioconazole by gavage

Parameter	Incidence (%)				
	Dose (mg/kg bw per day)				
	0	10	30	80	350
No. of fetuses evaluated	176	210	190	205	152
Unossified sternebra 5	13	12	16	18	20*
Unossified 13th rib	49	63**	65**	54	37*
Left forelimb:					
Incomplete ossification metacarpal 1	34	31	28	27	0**
Incomplete ossification digit 5 phalanx	56	58	64	48	28**
Unossified digit 5 phalanx	43	41	54	52*	72**
Right forelimb:					
Incomplete ossification metacarpal 1	34	31	28	28	0**
Incomplete ossification digit 5 phalanx	53	57	61	44*	27**
Unossified digit 5 phalanx	46	42	38	55*	72**
Left hindlimb:					
Incomplete ossification digit 4 phalanx	59	52	52	51	26**
Unossified digit 4 phalanx	14	17	12	22*	26**
Right hindlimb:					
Incomplete ossification digit 4 phalanx	59	50	51	50	26**
Unossified digit 4 phalanx	14	19	13	23*	26**
No. of litters examined	20	24	21	23	17
Left forelimb:					
Incomplete ossification metacarpal 1	95	67*	95	61**	0**
Incomplete ossification digit 5 phalanx	100	100	100	96	76*
Right forelimb:					
Incomplete ossification metacarpal 1	95	67*	90	61**	0*
Left hindlimb:					
Incomplete ossification digit 4 phalanx	100	96	95	96	76*
Right hindlimb:					
Incomplete ossification digit 4 phalanx	100	96	95	96	76*

From Becker & Biedermann (1998)

* $p < 0.05$, ** $p < 0.01$ (Fisher's exact test)

locomotor activity at appropriate intervals. These FOB and activity investigations were conducted before treatment and at 4 h and 7 and 14 days after treatment. After 14 days, all rats were killed and subjected to gross autopsy, when brain weights were recorded and whole-body perfusion fixation was performed on six males and six females per group. For rats in the control group and rats receiving the highest dose, central and peripheral nerve tissues and muscle tissue were examined microscopically. This study complied with GLP and with OECD guideline 424 (1997).

The stability and homogeneity of dosing solutions was determined by analysis.

There were no deaths. Perianal brown staining was recorded in male and female rats at ≥ 817 mg/kg bw, which resolved within 5 days. There were no effects on body weights. The only notable FOB findings were perianal brown staining and soft faeces at 4 h after treatment at doses of ≥ 817 mg/kg

bw. Motor activity and locomotor activity was reduced in males and females at 2240 mg/kg bw and in males at 817 mg/kg bw, but only at 4 h after treatment. There were no adverse effects on activity or FOB findings at 7 or 14 days after treatment. There were no notable gross observations at autopsy and no effects of treatment on brain weights. Microscopic examination of tissues from perfused rats of the group at 2240 mg/kg bw did not reveal any effects of treatment and examinations were not extended to lower doses.

The NOAEL was 218 mg/kg bw on the basis of transient clinical signs and a transient reduction in motor activity in male rats at 817 mg/kg bw (Sheets & Lake, 2000).

(b) Neurotoxicity

Groups of 12 male and 12 female Wistar rats were given prothioconazole (purity, 97.6–98.8%) at a nominal dose of 0, 100, 500 or 1000 mg/kg bw per day by gavage formulated in aqueous 0.5% methylcellulose and 0.4% Tween 80, 5 days per week for 13 weeks. Actual mean doses were 0, 98, 505 and 1030 mg/kg bw per day. Since these values were very close to the target values, the latter figures were used throughout this description. Investigations included clinical signs, body weights and food consumption, and FOB, motor and locomotor activity at 4-week intervals in compliance with OECD guideline 424 (1997), except for the absence of an investigation during weeks 1 or 2 (but compliant with the equivalent US EPA guideline). Ophthalmoscopy was also performed before treatment and in week 12. After 13 weeks, all rats were killed and subjected to a gross autopsy, at which brain weights were recorded and whole-body perfusion fixation was performed on six males and six females per group. For rats in the control group and rats at the highest dose, central and peripheral nerve tissues and muscle tissue were examined microscopically.

The stability and homogeneity of the dosing solutions was determined by analysis.

There were no treatment-related deaths. The only notable clinical sign recorded was a treatment-related occurence of urine staining of the fur in groups at 500 mg/kg bw per day and above. Oral staining was also recorded in three males and one female at 1000 mg/kg bw per day. During the first week of the study, body weight increased only slightly in the controls and the group at the lowest dose and decreased in the groups at the intermediate and highest dose. These differences resulted in differences in weight gain during the first week of 4–5% for males at 500 and 1000 mg/kg bw per day, compared with the controls. For the final 5 weeks of treatment, the difference in body weight for male rats in the groups at 500 and 1000 mg/kg bw per day averaged 5–7% and 7–9%, respectively, less than values for the controls. Food consumption was not affected by treatment and there were no notable ocular findings by ophthalmoscopy. FOB effects recorded at 500 and 1000 mg/kg bw per day were slight to moderate/severe urine staining. There were no other notable FOB findings. Reduced motor (males) and locomotor (males and females) activity was recorded at 1000 mg/kg bw per day.

At termination, the only notable finding on gross examination was increased incidence of wetness or staining of the ventrum in rats at 500 and 1000 mg/kg bw per day. There were no significant effects on brain weights; a slightly higher relative brain weight in males at the highest dose was a consequence of the lower terminal body weight. Microscopic examination of tissues from perfused rats at 1000 mg/kg bw per day did not reveal any effects of treatment and examinations were not extended to lower doses.

The NOAEL was 100 mg/kg bw per day on the basis of clinical signs (urine-staining of the fur) and reduced body-weight gain at 500 mg/kg bw per day. Reduced motor and locomotor activity at 1000 mg/kg bw per day was likely to be a consequence of systemic toxicity rather any specific effect on neurobehaviour (Sheets & Lake, 2001).

Since prothioconazole is not a member of a chemical class associated with inhibition of cholinesterase activity and no signs of toxicity consistent with the inhibition of cholinesterase activity have been recorded, no study of this parameter has been conducted.

Prothioconazole-desthio

Prothioconazole-desthio (M04) accounts for most of the residue found in wheat grain. It is only a minor systemic metabolite found in rats, dogs and goats. Prothioconazole-desthio appears to be toxicologically more potent than prothioconazole itself.

3. Biochemical aspects: absorption, distribution, excretion and kinetics

A pilot study (Klein, 1991; Koester, 2001) was terminated early, because the development of prothioconazole-desthio as an active ingredient was discontinued after a considerable data package had been compiled on its toxicological profile.

The absorption, distribution and excretion characteristics of the compound were studied only in male Wistar rats. The study was conducted using only [phenyl-UL-^{14}C]-labelled prothioconazole-desthio.

The radiochemical purity of [phenyl-UL-^{14}C]prothioconazole-desthio was 99.2%. The rats received single doses at 1 mg/kg bw or 5 mg/kg bw by gavage (for whole-body autoradiography).

After an oral dose, the prothioconazole-desthio was rapidly absorbed, the T_{max} calculated from concentrations of prothioconazole in the plasma being 1.5 h, at which time the C_{max} was 0.052 µg/g. This value corresponded to just 5% of the concentration per g bw, indicating a rapid distribution from the blood to the peripheral tissues. Since 90% of the administered dose was excreted via the bile and urine, it can be inferred that there was almost complete absorption from the gastrointestinal tract.

In two experiments, the mean concentrations of radiolabel in the body minus the gastrointestinal tract were 2.86% and 3.47% of the administered dose, indicating that there was little distribution to the peripheral tissues. In these, most radiolabel was found in the liver (3.044% of the administered dose) followed by the kidneys (0.042%) and erythrocytes (0.040%). Concentrations were very low in all other tissues and therefore did not indicate any potential for accumulation.

One hour after oral administration for whole-body autoradiography, the most intense autoradiography signal occurred in the gastrointestinal tract and liver, followed by the renal cortex. The concentration of radiolabel in the blood was lower than in fatty tissues, reflecting the lipophilicity of prothioconazole-desthio and, possibly, its metabolites. All other organs and tissues showed markedly less signal. The particularly intense signal in the intestinal contents, particularly at 4 h after dosing, indicated that extrabiliary secretion into the gastrointestinal tract had occurred. The general pattern of distribution of radiolabel did not alter during 48 h.

Plasma kinetics in pregnant rats

A study of comparative plasma kinetics was conducted in pregnant rats given prothioconazole by oral gavage or dermal application. An outline of the study is presented in Table 36.

The chemical and radiochemical purities of [phenyl-UL-^{14}C]prothioconazole-desthio were > 98%. Oral administration was by gavage, and dermal application was made on to shaved, intact dorsal skin (5 × 5 cm) under an occlusive dressing for 6 h per day. After removal of the occlusive dressings, the application sites were washed with warm water. The results are summarized in Table 37.

The time-to-maximum plasma concentration (T_{max}) was markedly prolonged by dermal application, but was independent of dose and treatment regimen. The maximum plasma concentrations were increased in an approximately 1 : 1 relationship to the doses administered by both routes. Also, maximum plasma concentrations (C_{max}) were comparable for oral and dermal doses and multiple oral doses of 1 mg/kg bw and multiple dermal doses of 30 mg/kg bw produced

Table 36. Outline of a study of plasma kinetics in pregnant rats given prothioconazole-desthio

Route of administration	Oral				Dermal			
Test No.	1	2	3	4	5[d]	6	7[d]	8
Dose (mg/kg bw)	1	1	3	3	30	30	100	100
No. of rats used	5	5	5	3[b]	5	3[b]	5	4[c]
No. of doses	1	9 + 1[a]	1	9 + 1[a]	1	9 + 1[a]	1	9 + 1[a]
Treatment period (days of gestation)	6	6–15	6	6–15	6	6–15	6	6–15
Duration of test (days)	2	12	2	12	2	12	2	12

From Weber (2001)

[a] Nine unlabelled doses + one final radiolabelled dose.

[b] Two rats not pregnant.

[c] One rat not pregnant.

[d] Tests repeated due to exaggerated radioactivity dose.

Table 37. Kinetic parameters in pregnant rats given radiolabelled prothioconazole-desthio as single or repeated doses administered oralyl or dermally

Parameter	Oral administration				Dermal administration			
	1 mg/kg bw		3 mg/kg bw		30 mg/kg bw		100 mg/kg bw	
	× 1	× 10	× 1	× 10	× 1	× 10	× 1	× 10
C_{max} (µg/ml)	0.066	0.065	0.211	0.258	0.036	0.054	0.087	0.242
T_{max} (h)	1.00	1.00	1.50	0.67	8.00	8.00	8.00	8.00
$T_{½}$ (h)	16.9	21.3	22.2	21.5	23.8	15.6	[34.4][a]	22.2
Intercept (µg/ml)	0.028	0.019	0.076	0.058	0.033	0.073	0.060	0.223
$AUC_{0-\infty}$ (µg.h/ml)	0.99	0.87	3.82	2.98	1.08	1.36	3.12	6.72
MRT (h)	18.2	21.8	22.2	20.7	35.3	26.3	49.4	34.6

From Weber (2001)

AUC, area under the curve of concentration–time; MRT, mean retention time.

[a] Low curve-fit correlation coefficient (0.77).

similar systemic doses. Mean retention times (MRT) were higher after dermal application, the difference being about 100% after single doses and 25–50% after multiple doses. Terminal half-lives were not affected by the route of administration and the area under the dose–response curve (AUC) for single and multiple doses were almost identical for oral and dermal administration (Weber, 2001).

Metabolism

Metabolites were identified only from a composite sample of bile since the isolation and purification of faecal metabolites was impractical owing to the large amount of matrix material present in the extracts in combination with the complex metabolic pattern. Also, approximately 40% of the total faecal radioactivity was unextractable. Urinary metabolites were not investigated as urinary excretion was a very minor route of excretion and consisted of much radioactivity derived from enterohepatic recirculation. In bile (0–24 h composite bile sample), 18 radioactivity peaks accounted for 84.4% of the administered dose, from which metabolites accounting for 39.6% of the administered dose were isolated and identified. Metabolism proceeds via oxidation reactions on the

phenyl moiety and the hydroxylated products were subsequently glucuronidated and methylated. The cyclopropyl and triazole rings of prothioconazole-desthio remain intact. No other metabolites were isolated because the study was prematurely terminated since development of prothioconazole-desthio as an active ingredient for formulation was discontinued (Koester, 2001). The metabolic pathway for prothioconazole-desthio is shown in Figure 1.

4. Toxicological studies

4.1 Acute toxicity

(a) Oral administration

Mice

The acute oral toxicity of prothioconazole-desthio (purity, 94.7%) was evaluated in groups of five male and five female fasted NMRI mice given a single dose at 100, 500, 1000, 2000, 2500, 3150, 4000 or 5000 mg/kg bw by gavage in 1% v/v aqueous Cremophor EL. The mice were observed for

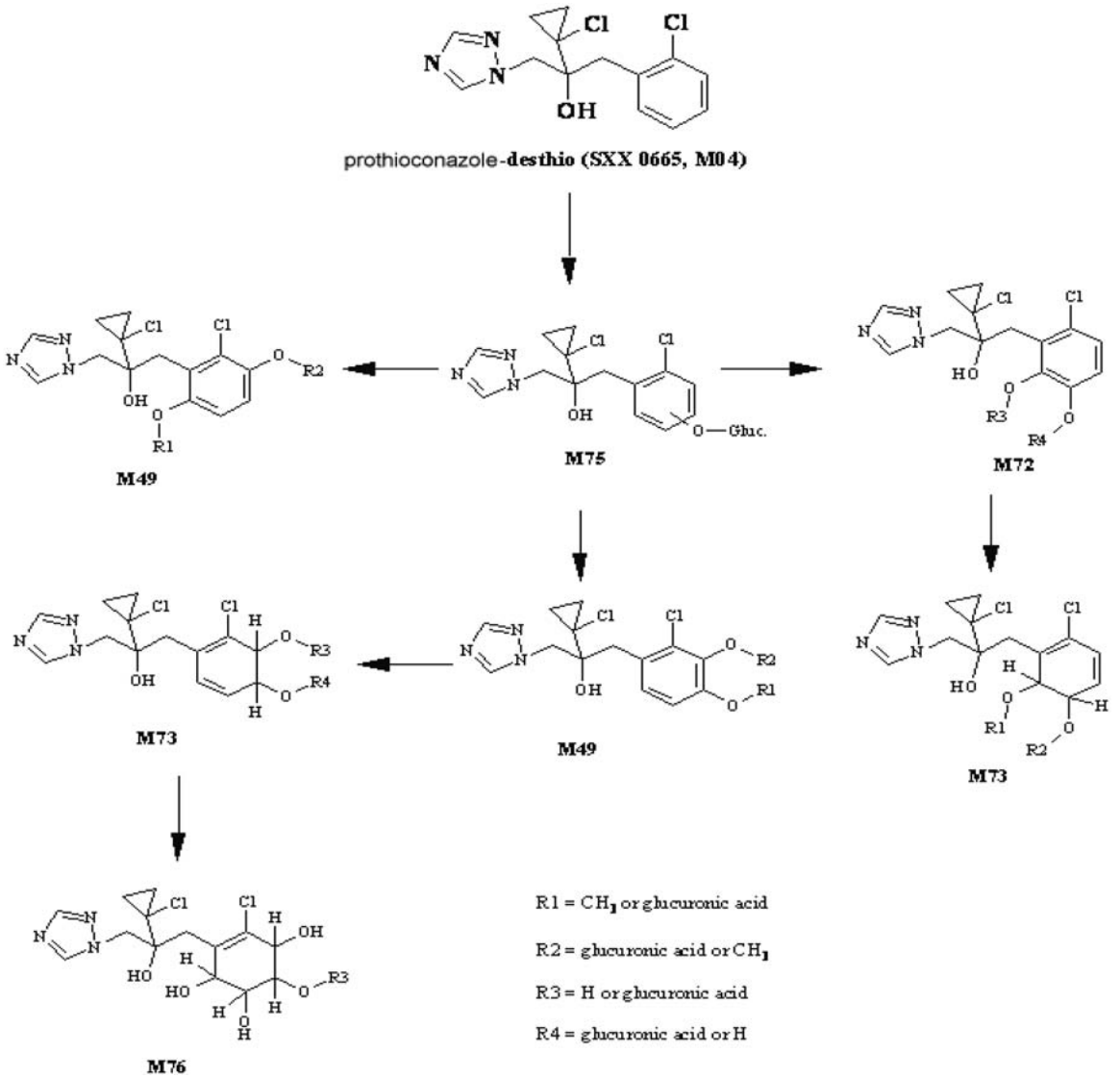


Figure 5. Metabolic pathways of prothioconazole-desthio in rats

14 days before autopsy except for females treated with 2000 and 3150 mg/kg bw, for which observation was extended to 21 days. Mortalities were delayed by 1–4 days and occurred at doses of 1000 mg/kg bw and higher in males and 2000 mg/kg bw and higher in females. Clinical signs of a reaction to treatment were recorded "several" times during the day of treatment and at least once daily thereafter during the observation period. The observed clinical signs (motility and respiratory disturbances, piloerection, staggering gait, narrowed palpebral fissures, lacrimation, a spasmodic state, temporary rolling over, prostration or lying on the side) were mainly of up to moderate intensity, developed shortly after treatment in some cases, and persisted at maximum up to the eleventh day of the study in the male mice or up to the seventh day in the females. The dose of 100 mg/kg bw was tolerated by male and female mice without clinical signs. The acute oral LD_{50} of prothioconazole-desthio in mice was 2235 mg/kg bw in males and 3459 mg/kg bw in females (Kroetlinger, 1991d).

Rats

The acute oral toxicity of prothioconazole-desthio (purity, 93.7%) was evaluated in groups of five male and five female fasted Wistar rats given a single dose at 100, 500 (males only), 1000 (females only) 2000, 2500, 3150 or 4000 mg/kg bw by gavage in 1% v/v aqueous Cremophor EL. The rats were observed for 14 days before autopsy, except for females at 2000 and 3150 mg/kg bw, for which observation was extended to 21 days.

Mortalities were delayed by 4–13 days and occurred at doses of 2500 mg/kg bw and higher in male rats and 2000 mg/kg bw and higher in female rats. Clinical signs of a reaction to treatment were recorded "several" times during the day of treatment and at least once daily thereafter during the observation period. Clinical signs of response to treatment were noted in males and females at doses higher than 100 mg/kg bw. Consistent clinical signs comprised apathy, piloerection, laboured breathing, staggering gait and increased urination in males at 500 mg/kg bw and in females at 1000 mg/kg bw. Spastic gait and reduced mobility were also noted in females at doses greater than 100 mg/kg bw, but these signs were noted in males only at doses of greater than 2000 mg/kg bw. Atony, weak reflexes, emaciation, pallor, narrowed palpebral fissures (separation between the upper and lower eyelids), red crusted eyelids, bloody snout, prone position and leg extension occurred in males and females at higher doses. Some clinical signs were evident shortly after treatment, but others showed a delayed onset. All symptoms had resolved by day 13 in male rats and by day 18 in females. Body-weight gains were markedly retarded or there was even weight loss in rats of males and females treated at doses greater than 2000 mg/kg bw, particularly during the first week of observation. Body-weight gains were unaffected by treatment among females treated at 100 mg/kg bw and males treated at 100 and 500 mg/kg bw. Consistent gross pathology findings in decedents treated at doses greater than 2500 mg/kg bw comprised mottled appearance of the lungs with or without slight to severe distension, pale mottled and/or lobulated appearance of the liver with or without slight organ enlargement, slight to marked reddening of the glandular stomach, frequently accompanied by ulcer-like foci or crateriform elevations, and pale or patchy appearance of the spleen and kidneys. There were no gross lesions at autopsy in surviving rats treated at up to 2500 mg/kg bw, with the exception of one male at 2500 mg/kg bw that showed a slightly enlarged and discoloured liver. The acute oral LD_{50} for prothioconazole-desthio was 2806 mg/kg bw in male rats and 2506 mg/kg bw in female rats (Kroetlinger, 1991a).

(b) Dermal administration

Rats

The acute percutaneous (dermal) toxicity of prothioconazole-desthio (purity, 93.7%) was investigated in groups of five male and five female Wistar rats given the test material as a single dose at 5000 mg/kg bw (equivalent to 29.7–32.9 mg/cm^2) as a paste in physiological saline to the shaved skin under a semi-occlusive dressing for 24 h. After the exposure period, the treated area was cleaned with soap and water. The rats were observed for 14 days and then killed and subjected to autopsy.

There were no deaths and no systemic or local clinical signs of toxicity. The acute dermal LD_{50} was therefore > 5000 mg/kg bw (Kroetlinger, 1991b).

(c) Inhalation

A test for the acute toxicity of prothioconazole-desthio administered by inhalation was conducted in groups of five male and five female Wistar rats that received nose-only exposures for 4 h either to a dust (purity, 95.4%) at analytically determined concentrations of 0.52 or 5.08 mg/l (particle MMAD, 13–40 μm), or to an aerosol (purity, 93.7%; in a 1 : 1 PEG 400/ethanol vehicle) at an analytically determined concentration of 0.26 mg/l (particle MMAD, 1.5 μm). A 1 : 1 PEG 400/ethanol vehicle control was also included. Analysis of particle-size distribution demonstrated that a high mass fraction of the aerosol was respirable, while neither concentration of the dust was within the readily-respirable size range.

No deaths occurred and there were no treatment-related clinical signs evident in any of the test groups. Similarly, there were no treatment-related effects observed in a battery of reflex assessments made on the first day after exposure. Minimal and transient weight loss occurred during the first 3 days after exposure in males and females exposed to dust at 5.08 mg/l. At autopsy, there were no treatment-related gross lesions observed in any of the rats. The acute LC_{50} of respirable-particle size prothioconazole-desthio in rats was > 0.26 mg/l. The acute LC_{50} of prothioconazole-desthio dust particles greater than respirable size in rats was > 5.08 mg/l (Pauluhn, 1992).

(d) Intraperitoneal injection

A test for the acute toxicity of prothioconazole-desthio (purity, 94.0%) formulated in 1% Cremophor EL in physiological saline and administered intraperitoneally was conducted in groups of five male and five female Wistar rats in astudy that complied with OECD guideline 401. Males received doses of 10, 100, 355, 400, 450, 500 or 800 mg/kg bw and females received doses of 10, 100, 500, 800 or 1000 mg/kg bw.

Deaths occurred in males and females at doses of greater than 500 mg/kg from day 1 to day 6 of the observation period. Clinical signs were recorded at all doses, with the exception of 10 mg/kg bw. Males and females treated at 100 mg/kg bw showed apathy and laboured breathing. Decreased mobility, staggering gait and atony were also apparent in males at 100 mg/kg bw, but occurred in females only at doses of 500 mg/kg bw and higher. Piloerection, poor or no reflexes, prostration or lying on side, spasms, transient convulsions, stretched legs and lacrimation were observed in males and females at higher doses. Narrowed palpebral fissures, soft faeces, increased urine excretion, emaciation, splayed hind limbs and dull corneas were also occasionally observed. Clinical signs were generally evident within a few minutes of treatment and persisted for up to 75 min at 100 mg/kg bw, while at higher doses some clinical signs persisted for up to 11 days in males and up to 4 days in females. Body-weight gain was generally unaffected by treatment at 10 and 100 mg/kg bw. At higher doses in males and females, transient body-weight loss occurred during the 4 days after treatment. Consistent, gross pathological findings considered to be treatment-related were distended, patchy lungs in decedents of males and females at doses of greater than 500 mg/kg bw, and pale/patchy and lobulated liver in decedents and some surviving males treated at doses greater than 355 mg/kg bw. The acute intraperitoneal LD_{50} was estimated to be 450–500 mg/kg bw for male rats and approximately 632 mg/kg bw for female rats (Kroetlinger, 1991c).

(e) Dermal irritation

Prothioconazole-desthio technical (purity, 95.4%) was evaluated for acute dermal irritation potential in three female New Zealand White rabbits. Prothioconazole-desthio (0.5 g) was moistened with water and applied to a 6 cm^2 gauze pad that was placed on the shaved dorsal area. The gauze patch was covered with plastic, secured with tape and left for 4 h. Thereafter, patches were

removed and the test sites were washed using tap water and paper towels. Adjacent areas of untreated skin of each rabbit served as controls for the test. Skin was examined 1 h, 24 h, 48 h, 72 h and 7 days after dosing. Dermal irritation was scored and recorded according to the Draize technique. All rabbits were scored zero for both erythema/eschar formation and oedema at all examination times (Märtins, 1991).

(f) Ocular irritation

Prothioconazole-desthio technical (purity, 95.4%) was evaluated for acute ocular irritation potential in three female New Zealand White rabbits. Each rabbit received 45 mg of prothioconazole-desthio powder into the conjunctival sac of the right eye after gently pulling the lower lid away from the eyeball. The lid was then gently held together for about 1 s in order to prevent loss of test material. The left eye, which remained untreated, served as a control. After 24 h, the eyes were washed with saline. The eyes were examined at 1, 24, 48 and 72 h after washing. At 24 h after washing, the eyes were treated additionally with fluorescein and examined.

All rabbits displayed ocular discharge at 1 h, but not at any later time-point, and two rabbits showed slight (grade 1) conjunctival redness at 24, 48 and 72 h. No ocular reactions were apparent at 7 days. The group mean irritation indices for the observations at 24, 48 and 72 h were 0.0 for corneal opacity, iridial damage and conjunctival chemosis, and 0.67 for conjunctival redness (Märtins, 1991)

(g) Dermal sensitization

The potential of prothioconazole-desthio (purity, 94.7%) to produce dermal sensitization in female Dunkin Hartley strain guinea-pigs using the Buehler patch test was investigated. Twelve guinea-pigs were used for the treatment group, while current guidelines stipulate 20. The concentration of prothioconazole-desthio for topical induction and challenge applications in the main study was the maximum technically achievable concentration of 60%. This concentration was selected on the basis of a range-finding study in which no skin reactions occurred at any of the test concentrations tried (12%, 25%, 50% and 60%).

A group of 12 male guinea-pigs received three topical induction applications of 0.5 ml prothioconazole-desthio formulated at 60% in sterile physiological saline and 1% Cremophor EL. The applications were made at weekly intervals to a shaved area of left flank skin and covered with a hypoallergenic patch and retained in place for 6 h with adhesive tape. A group of 12 guinea-pigs similarly treated, but with vehicle alone, acted as a control group. After each 6-h topical application, the test material was washed from the skin sites with physiological saline. The application sites were assessed for skin reactions 24 h after patch removal. A topical challenge dose of 0.5 ml of 60% prothioconazole-desthio was applied for 6 h under a hypoallergenic patch and adhesive tape to the shaved left flank of both test and control groups. Similar patches wetted with 0.5 ml of vehicle only were applied to the right flanks of both test and control guinea-pigs. On removal of the patches after 6 h, the challenge sites were chemically depilated and the skin reactions were graded for erythema on a five-point scale 24, 48 and 72 h after the initiation of the challenge. Clinical observations were recorded daily and individual body weights were recorded pretest and at termination on day 31. The dermal responses of the group receiving prothioconazole-desthio were compared with the intensity and duration of the responses in the control group.

There were no deaths and no clinical signs of a systemic reaction to treatment in either the test or control groups. Body-weight gain of the test group was comparable to that of the controls. No skin reactions developed in either the test or control groups after induction or challenge applications. Consequently, skin reaction grades were zero for all test and control guinea-pigs at all time-points. The incidence of sensitization was zero in the test group. Contemporary positive controls conducted at the same laboratory provided satisfactory results (Dreist & Diesing, 1991).

4.2 Short-term studies of toxicity

(a) Oral administration

Mice

Groups of 10 male and 10 female B6C3F$_1$ mice were fed diets containing prothioconazole-desthio (purity, 93.7%) at a concentration of 40, 200, 1000 or 5000 ppm for 13 weeks. These dietary concentrations were equal to measured doses of 0, 11.5, 58.9 and 294 mg/kg bw per day in male mice and 0, 16.0, 79.5 and 392.3 mg/kg bw per day in female mice. Haematology and blood chemistry investigations were performed at the end of the study. A gross autopsy was performed on all mice and brain, liver, spleen, kidneys, adrenal glands and testes were weighed. After autopsy, the activities of ECOD, EROD, ALD, EH, GST and GLU-T were measured in liver samples from five males and females per dose. Histopathology was performed on the liver of mice from all groups, all gross findings and selected organs from mice in the control group and group at the highest dose only. Sections of preserved tissues from all mice at 0 or 1000 ppm including oil red O-stained liver cryocuts, and liver, spleen, ovaries and gross lesions from all mice, including those at 5000 ppm, were examined by light microscopy.

Treatment-related mortality was confined to the group treated at 5000 ppm. All mice either died or were killed when in a moribund condition during the first week of treatment. Before death, the mice adopted a squatting position and showed apathy and poor general condition. A male mouse treated at 40 ppm died in an emaciated condition, but no other mice treated at up to 1000 ppm showed any clinical signs of an adverse reaction to treatment. There were no treatment-related ophthalmological findings at 1000 ppm. The overall body-weight gains of males and females at 1000 ppm and males at 200 ppm were reduced by up to 71%. The effect was less severe in females at 1000 ppm (32% reduction) and in males at 200 ppm (26% reduction). Food consumption in contrast was unaffected by treatment at any dose.

Many haematological parameters in male mice were significantly affected by treatment at 1000 ppm, but not at lower doses. In the male mice at 1000 ppm, there were reductions in erythrocyte counts, erythrocyte volume fraction, MCV, MCHC, platelet counts and leukocyte counts. The haematology of female mice was not adversely affected at any dose after 14 weeks of treatment.

The plasma activity of GLDH was statistically significantly increased in males and females at 1000 ppm by 192% and 181%, respectively, and was higher, but not significantly different from the controls in males at 200 ppm. The activities of AST, ALT and ALP also were statistically significantly increased in males at 1000 ppm by 54%, 84% and 74%, respectively. ALT was also higher, but not significantly so, in females of the group at 1000 ppm. Cholesterol concentrations were statistically significantly reduced and triglyceride concentrations were statistically significantly increased in males and females at 1000 ppm. Plasma albumin concentrations were statistically significantly reduced in males at 200 and 1000 ppm by about 8%, while plasma total protein was unaffected by treatment. Total bilirubin concentration was reduced in males by 26% at 1000 ppm. All other clinical-chemistry parameters were unaffected by treatment at up to 1000 ppm.

There were statistically significant increases in the hepatic activity of ECOD in males and females at 200 ppm by 173% and 81%, respectively, and 1000 ppm by 460% and 225%, respectively. The activity of ALD was also statistically significantly increased in males and females at 40 ppm by 121% and 38%, respectively, 200 ppm by 376% and 189% and 1000 ppm by 707% and 302%, respectively. EROD was statistically significantly increased in all treated males, but without any dose–response, and in female mice at 200 ppm and 1000 ppm by 50% at both doses. In addition, liver GST activity was increased in females at 1000 ppm by 159%. EH and GLU-T enzyme activities were unaffected by treatment with prothioconazole-desthio.

Observations of treatment-related gross lesions at autopsy were confined to the mice of the group at 5000 ppm that died or were killed because of their moribund condition. These were: abnormal contents of the small and/or large intestine and areas of change in the glandular mucosa of the stomach. All other gross lesions were considered to be incidental to treatment with prothioconazole-desthio. Absolute and relative liver weights were statistically significantly increased by 5–14% in males at 200 ppm and by 36–85% in males and females at 1000 ppm. The absolute spleen weights were statistically significantly increased by 11% and 21% in males at 200 and 1000 ppm, respectively, and relative weights were statistically significantly increased by 15% and 29%, respectively. Since there were no relevant haematological effects or histomorphological correlates in the tissues of the haematopoietic system of males at 200 ppm, the increased spleen weight at this dose was of uncertain significance. Although absolute weights of the testis and kidney in male mice at 1000 ppm were statistically significantly lower than those of the controls, these effects were considered to be secondary to the lower body weights at this dose, because the relative weights of these organs were unaffected. All other organ weights were unaffected by treatment at any dose.

Treatment-related histopathological alterations occurred in the liver of decedents at 5000 ppm. Hepatocyte hypertrophy occurred in males at 200, 1000 and 5000 ppm and in females at 40, 200 and 1000 ppm (none recorded in female decedents at 5000 ppm). Periacinar hepatocytic fatty vacuolation, single-cell or focal necrosis, apoptosis, hydropic degeneration and increased ploidy occurred in a proportion of males and females at 5000 ppm. Focal necrosis and hepatocytic fatty vacuolation in males and single-cell or focal necrosis and hydropic degeneration in females also occurred at 1000 ppm. Periacinar hepatocytic fatty vacuolation and single-cell necrosis was also observed in some females at 200 ppm. In addition, treatment-related histopathological alterations occurred in the spleen and ovaries, and in the stomach of decedents at 5000 ppm. In the spleen, follicular atrophy, hypocellularity of the red pulp and the presence of large, pigment-laden macrophages occurred in males and females at 5000 ppm. There was a dose-related increase in the incidence and severity of haemorrhagic degeneration of the corpora lutea at doses of 200 and 1000 ppm. Focal erosion, observed in mice at 5000 ppm, with changed areas of glandular mucosa in the stomach, is indicative of local irritation.

A NOAEL could not be identified owing to the occurrence of treatment-related microsomal enzyme induction and hepatocyte hypertrophy at the lowest dose administered, 40 ppm, equal to 12 and 16 mg/kg bw per day, in males and females respectively (Wirnitzer, 1999).

Rats

Groups of 10 male and 10 female Wistar rats were given diets containing prothioconazole-desthio (purity, 93.7%) at a concentration of 0, 100, 300 or 1000 ppm, equal to 0, 11, 34 and 117 mg/kg bw per day for male rats and 0, 11, 38 and 121 mg/kg bw per day for female rats, for 28 days. The stability of prothioconazole-desthio in the diet and the homogeneity of the dietary mixtures were verified before the start of the study. Analyses for correct concentration were performed before the study start. Food consumption and body weight were determined once per week. The state of health of the rats was checked twice per day. Eye examinations were carried out on five males and five females per group before the start and at the end of the dosing period. Blood samples were taken from all rats for haematology and blood chemistry examinations at the end of the dosing period. In addition, serum T3, T4 and T4 binding capacity (TBC), were analysed in five males and five females per group. 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. Microscopic examinations were conducted on liver and all gross lesions in rats from all groups receiving prothioconazole-desthio. Additional liver samples were retained from five males and five females per group for the determination of hepatic cytochrome P450, aminopyrine-*N*-demethylase and *p*-nitroanisole-*O*-demethylase activities and triglyceride concentration. Sections of all preserved tissues from five males and five females at 0

or 1000 ppm including oil red O-stained freeze-sectioned liver and PAS-stained kidney sections, and liver, ovary, thyroid and gross lesions from all rats were examined by light microscopy.

There were no mortalities in the study and prothioconazole-desthio did not induce any remarkable clinical signs of toxicity in any of the groups. Body-weight gain, and food and water consumption were unaffected by treatment.

Ophthalmoscopy revealed no treatment-related findings. There were no toxicologically significant effects on the haematological profiles of rats at any dose, although there were some minor differences from the controls. The group mean MCH value for males at 1000 ppm was statistically significantly lower than the value for controls, but the magnitude of the effect was < 5% and occurred in the absence of any other indication of erythrocyte damage. The number of circulating platelets was statistically significantly lower in males at 1000 ppm than in the controls, but was within the normal range. Females at this dose showed lower relative monocyte counts than the controls; however, this is not an unusual occurrence in isolation.

Treatment-related, statistically significant changes in blood-chemistry parameters for rats at 1000 ppm included reductions in total bilirubin by 22% and triglyceride by 44% in males and an elevation in cholesterol by 46% in females. Triglyceride concentrations were also higher by 44% in females at 1000 ppm, but not significantly so. Blood creatinine concentrations were reduced at 1000 ppm by 16% in males and 13% in females. Circulating T4 concentrations were significantly lower at 1000 ppm in male rats by 20%, but not in females, and neither T3 concentrations nor TBC was affected by treatment. During urine analysis, blood and erythrocytes were observed in the urine of one male and one female at 1000 ppm. In the affected male rat, urinary bilirubin was also detected. There were no other treatment-related effects on urine-analysis parameters.

Absolute liver weights were statistically significantly increased by about 27% and 25% in males and females at 1000 ppm, respectively, and by about 16% in males at 300 ppm. Relative (to body weight) liver weights also were significantly increased at these doses and additionally at 100 ppm by about 5% in males and 7% in females. Absolute and relative kidney weights were statistically significantly increased at 1000 ppm, in female rats, 8% and 12.5%, respectively. There was a dose-related increase in ovary weights that were statistically significant at all doses. At 100 ppm, the increases in absolute and relative ovary weights were 33% and 31%, respectively. Again in female groups only, there were statistically significant increases in absolute and relative weights of the adrenal at 300 and 1000 ppm and in the absolute weight of the pituitary at 100 and 1000 ppm. These weight changes in the adrenal and pituitary were not clearly an effect of treatment in view of the absence of a dose-related response.

Hepatic tissue activities of P450 and *p*-nitroanisole-*O*-demethylase were statistically significantly elevated in males and females at 1000 ppm. P450 activity was also statistically significantly elevated in males at 300 ppm. Aminopyrine-*N*-demethylase activity increased at 1000 ppm in males and females, but not significantly so. These effects indicate treatment-induced induction of hepatic enzymes. Hepatic triglyceride concentration was elevated in males and females at 300 and 1000 ppm.

At autopsy, no treatment-related gross lesions were found in male rats at any dose. However, female rats in all treatment groups had enlarged ovaries with follicles filled with clear, watery fluid. No other treatment-related gross findings occurred.

Microscopic examination indicated that there was a treatment-related occurrence of minimal to moderate fatty changes in the livers of males treated at any dose. The severity of the effect generally increased with increasing dose and was characterized by predominantly mid-zonal or periportal intracytoplasmic inclusion of microvesicular fat. Female rats also showed an increased incidence of the change at all doses, but the severity of the change was comparable to that of the two females in the control group in which fatty change was also observed. As a correlate to the gross findings in the ovary, females at 300 or 1000 ppm showed a treatment-related increased incidence of tertiary follicles

in the ovary. The granulosa layer of these follicles also appeared to be more irregular. The effect was accompanied by an increased incidence of stromal oedema. These findings were not confirmed in the 90-day study in rats (decribed below) below and are therefore of questionable toxicological relevance. No treatment-related histopathological lesions occurred in the adrenals, pituitary, thyroid gland or kidneys of either sex at 1000 ppm, to account for observed effects on organ weights or circulating thyroxine levels. There were no treatment-related microscopic lesions at 1000 ppm in any other tissues examined.

No NOAEL could be identified in this study in view of the observation of ovarian weight and histological changes in the liver at all doses, including 100 ppm, equal to 11 mg/kg bw per day, in male and female rats (Kroetlinger & Hartmann, 1992).

Groups of 10 male and 10 female Wistar rats were given diets containing prothioconazole-desthio (purity, 92.8%) at a concentration of 0, 30, 125, 500 or 2000 ppm, equal to 0, 2.2, 9.6, 36.9 and 161.9 mg/kg bw per day for male rats and 0, 3.0, 12.5, 50.7 and 210.8 mg/kg bw per day for female rats, for 14 weeks. An additional two groups of 10 males and 10 females were given diets containing prothioconazole-desthio at a concentration of 0 or 2000 ppm, equal to 0 and 162.2 mg/kg bw per day for male rats and 0 and 219.1 mg/kg bw per day for female rats for 14 weeks and then maintained for an additional 5 weeks without further treatment. Food consumption and body weight were determined once each week. The state of health was checked twice per day. Eye examinations were carried out on five males and five females per group before the start and at the end of the dosing period. Blood samples were taken from all rats for haematology and blood-chemistry examination at the end of the dosing period. In addition, serum T3 and T4 were analysed in five males and five females per group during weeks 5 and 14. Urine was analysed from all rats during weeks 4 and 13 and from the recovery groups during weeks 4 or 5 of the recovery period. All rats were subjected to complete gross examinations, and weights of selected organs were determined. Microscopic examinations were conducted on liver and all gross lesions in rats from all groups receiving prothioconazole-desthio. Additional samples of liver were retained from all rats for the determination of hepatic cytochrome P450, aminopyrine-*N*-demethylase and *p*-nitroanisole-*O*-demethylase activities and triglyceride concentration. Sections of all preserved tissues from all rats at 0 or 2000 ppm, including oil red O-stained freeze-sectioned liver and liver, kidney lung, ovary and gross lesions from all rats were examined by light microscopy.

There were no treatment-related mortalities in the study although two females (one each from the groups at 0 and 2000 ppm) died as a result of blood-sampling errors. Prothioconazole-desthio did not induce any remarkable clinical signs of toxicity in any of the groups and ophthalmoscopy revealed no treatment-related findings. A treatment-related reduction in overall weight gain of 13% and 12% occurred during the treatment period in the males and females at 2000 ppm, respectively. Over the same period there was a more marked reduction in body-weight gain of 22% in males and females of the recovery groups treated at 2000 ppm. These reductions in weight gain were in the absence of reductions in food consumption and were thus considered to be a result of systemic toxicity caused by the test material. Weight gain at lower doses was unaffected by treatment. During the recovery period, the body-weight gains of rats at 2000 ppm were comparable to or exceeded those of rats in the control group. Food consumption was unaffected by treatment at any dose. The reduction in food utilization led to significantly reduced protein and increased ketone concentrations in the urine of males at 2000 ppm in week 4. All other physicochemical characteristics and cellular and chemical constituents of the urine were unaffected by treatment at any dose.

The haemoglobin concentration and erythrocyte volume fraction values of males treated at dietary concentrations of 125 ppm and higher were statistically significantly reduced at week 5, but by no more than 6%. The haemoglobin concentration of females was not significantly affected by treatment and erythrocyte volume fraction values were significantly reduced at 500 and 2000 ppm by

7% and 9%, respectively. Since these effects at week 5 were small, not found at week 14 and there were no histomorphological alterations in the haematopoietic tissues examined, they were considered to be of no toxicological significance. The platelet counts were statistically significantly reduced at 2000 ppm in week 14 in males and females by 13%. It was not clear, however, that the observation was treatment-related, the platelet counts in males of the group at 30 ppm also being significantly reduced, by 11%, while there was no significant reduction at the intervening doses of 125 and 500 ppm. There was prolongation of the thromboplastin time in males at 500 and 2000 ppm in week 5 and at 2000 ppm in week 14, although these data also appeared to be erratic and not clearly a result of treatment. Thus, in females of week 14, there was no prolongation of the thromboplastin time at 2000 ppm (where there was a reduction in platelets), while there were significant increases in thromboplastin time at 30 and 500 ppm (where there were no reductions in platelets). There were no significant effects on haematology in the 5-week recovery group. There were no other relevant haematological findings at any dose.

In male rats of the group at 2000 ppm there were statistically significant increased plasma activities of AST, ALT, ALP and GLDH in week 5 by 17%, 29%, 24% and 825%, respectively. Only ALT activity remained significantly increased, by 28%, in the group of males at 2000 ppm at week 14. Although ALT activity was also increased, by 34%, in females of the group at 2000 ppm at week 14, it was noted that while there was a 27% increase in activity of this enzyme in the group of females at 30 ppm at week 14, there was no significant increase in groups of females at either 125 or 500 ppm at this time. The Meeting also noted that the large increase in GLDH activity in males at 2000 ppm at week 5 was mirrored by a large, but non-significant 90% decrease in activity in females of the same group and sample time and that there was no increase in activity of this enzyme in male rats of the group at 2000 ppm in week 14. However, GLDH activity was significantly reduced, by 74%, in female rats at 2000 ppm at the end of the recovery period. No effects on other plasma enzyme activities were apparent at the end of the recovery period. Triglyceride concentrations were statistically significantly decreased in male rats at week 5 in the groups at 500 and 2000 ppm, by 34% and 84%, respectively, and in males at week 14 in the group at 2000 ppm, by 70%. Serum triglyceride concentrations were reduced, by 39%, in females at 2000 ppm at the end of the recovery period. Cholesterol concentration was increased in females at weeks 5 and 14 in the group at 2000 ppm, by 38% and 25%, respectively, but not at the end of the recovery period. Total serum bilirubin concentration was statistically significantly reduced in the male groups at 500 and 2000 ppm and at all doses in females at week 14, but not at the end of the recovery period. These blood-chemistry effects may indicate a perturbation in liver function or may be a product in part of reduced food efficiency.

T4 concentration was statistically significantly reduced in males at 500 and 2000 ppm by 25% and 30%, respectively, at week 5 and by 14% (not statistically significant) and 19%, respectively, at week 14. There were no changes in T4 concentrations in female rats during the dosing period, while their T4 concentration increased by 14% in the group at 2000 ppm at the end of the recovery period. The serum concentrations of T3 were apparently unaffected by treatment (although there were statistically significant increases restricted to males at 30 and 125 ppm in week 14), while at the end of the recovery period the T3 concentration was statistically significantly increased by 18% in males at 2000 ppm. Since there were no accompanying changes in thyroid histology at any dose, the observed reductions in circulating T4 concentrations, and also the reduction in total bilirubin concentration, are probably related to the altered liver-enzyme activity profile.

There was a treatment- and dose-related increase in the absolute and relative liver weights of males and females at 500 and 2000 ppm. The increases at 2000 ppm were statistically significant, but at 500 ppm only the relative liver weight increase of the females was statistically significant. At the end of the recovery period, the liver weights were comparable to, or slightly lower than, values for the controls. After 14 weeks of treatment, there was a treatment-related increase in absolute and relative ovary weights at 2000 ppm that was not observed at the end of the recovery period.

Treatment-related gross lesions observed during autopsy were confined to pale or enlarged livers in some males at 500 and 2000 ppm and enlarged livers in some females at 2000 ppm. There were no similar observations recorded in rats in the recovery groups. Treatment-related microscopic alterations were confined to the liver. Hepatocellular hypertrophy, vacuolation and mid-zonal and centrilobular fatty change occurred at higher incidences and greater severity in males at 125 ppm and higher doses. Hepatocellular hypertrophy also occurred at higher incidence and severity in females at 500 and 2000 ppm than in controls. Hepatocyte vacuolation and diffuse or mid-zonal or centrilobular fatty change each occurred in three females at 2000 ppm., but were not observed in females at 500 ppm. The occurrence of hepatocellular hypertrophy, in isolation, is considered to be an adaptive sequel to hepatic-enzyme induction. Hepatocellular hypertrophy and mid-zonal fatty changes persisted throughout the recovery period in males at 2000 ppm, but no hepatic lesions were apparent at this time in the female rats. No other treatment-related histological lesions were recorded for any organ. In the ovaries, the numbers of corpora lutea and tertiary follicles were comparable in all groups and there was no histomorphological correlate for the increased ovary weight observed at 2000 ppm. In addition, there was no indication of any other endocrine influence in the pituitary or adrenal glands, therefore the increase in ovary weights is considered to be incidental and related to the normal different phases of the estrous cycle.

Results of biochemical measurements on liver homogenates showed that triglyceride content was clearly and statistically significantly increased by 275% in males of the group at 2000 ppm at week 15. The concentration was also greater, but not significantly so, by 88% in males at 500 ppm. Triglyceride content was statistically significantly greater at all doses in female rats, but these increases were nevertheless small in comparison with those found in males and may simply be an artefact of a low concentration of triglyceride in the control group. The increases in females were 25%, 38%, 37% and 46% in the groups at 30, 125, 500 and 2000 ppm, respectively. There was no significant difference in triglyceride concentrations between the groups at 0 and 2000 ppm at the end of the recovery period. The trigyceride concentration at the highest dose correlated with the relatively high severity score for mid-zonal and centrilobular fatty change that was observed in all male rats at 2000 ppm. While there was also a high severity score for females of this group, the observation was confined to a single animal. Cytochrome P450 content of the liver was significantly increased in male rats by 41% and 290% at 500 and 2000 ppm, respectively, and in female rats by 19%, 38% and 164% at 125, 500 and 2000 ppm, respectively. Although there were a number of statistically significant differences recorded for hepatic *p*-nitroanisole-*O*-demethylase and aminopyrine-*N*-demethylase activities, it is not clear what biological significance could be attached to them, except for the 70% and 100% increases observed in *p*-nitroanisole-*O*-demethylase activities in male and female rats, respectively, in the group at 2000 ppm. There may also be biological consistency in the small increases in aminopyrine-*N*-demethylase activity in female rats of 31% and 39% at 500 and 2000 ppm, respectively. However, the reduced activity of this enzyme recorded for all doses in male rats is inconsistent with the measurements of cytochrome P450 content (Table 38). At the end of the recovery period, there was a persistent elevation in *p*-nitroanisole-*O*-demethylase activity of 35% in male rats of the group at 2000 ppm, but there were no other significant differences between groups.

The NOAEL was 30 ppm, equal to 2.2 and 3.0 mg/kg bw per day in male and female rats, respectively, on the basis of microscopic alterations in the liver of males (hypertrophy, vacuolation and fatty change) 125 ppm, equal to 9.6 and 12.5 mg/kg bw per day in males and females, respectively (Schladt & Hartmann, 1999).

(b) Inhalation

Rats

Groups of 10 male and 10 female Wistar rats were exposed by inhalation to prothioconazole-desthio (purity, 95.4%) at analytically determined atmospheres of 0, 0.011, 0.054 or 0.235 mg/l in

Table 38. Certain enzyme activities and substrates measured (before and after recovery period) in liver homogenates of rats receiving diets containing prothioconazole-desthio for 14 weeks

Enzyme activity or substrate	Week	Males					Females				
		Dietary concentration (ppm)					Dietary concentration (ppm)				
		0	30	125	500	2000	0	30	125	500	2000
O-Dem (mU/g)	15	10	6**	7**	11	17**	8	6*	8	9*	16**
	20	8.1				10.9**	10.2				10.1
N-Dem (mU/g)	15	134	83**	86**	92**	82**	54	58	57	71*	75**
	20	113				127	63				70
P450 (nmol/g)	15	39	35*	37	55**	152**	36	39	43*	50**	95**
	20	36				39	32				36

From Schladt & Hartmann (1999)

O-Dem, *p*-nitroanisole-*O*-demethylase; N-Dem, aminopyrine-*N*-demethylase.

* $p < 0.05$; ** $p < 0.01$

nose-only chambers, for 6 h per day for 5 days. The atmospheres of prothioconazole-desthio were generated as aerosols in 1 : 1 PEG 400/ethanol (0.20 ml/l, MMAD, ≤ 1.25 µm ± GSD 1.39 µm). A control group was exposed to 1 : 1 PEG 400/ethanol only. The actual aerosol concentrations to which the rats were exposed were measured analytically by HPLC in samples withdrawn from the breathing zone at the start, middle and end of each exposure period. Five males and females per group were killed and autopsied on day 7 and the remaining rats were killed and autopsied after a 2-week treatment-free period. Clinical signs were recorded several times on the days of exposure and twice per day thereafter. Clinical examinations on the fourth day of exposure included subjective evaluation of visual placing response, grip-strength on wire mesh, abdominal muscle tone, corneal and pupillary reflexes, pinnal reflex, righting reflex, tail-pinch response and startle reflex. Individual body weights were recorded just before exposure and on days 4 and 7, and weekly thereafter. Rectal temperatures were recorded for five males and five females per group before dosing, immediately after exposure on day 4 of dosing and on day 7. Haematology and blood chemistry analyses, including T3, T4 and TBC, were performed on blood samples from five males and five females per group on day 7. All rats killed on day 7 and those killed on day 21 after the 2-week observation period were subjected to autopsy and post-mortem examination of major organs and tissues. Organ weights were recorded from the rats killed on day 7 and samples of liver were retained frozen for the measurement of the activities of aminopyrine-*N*-demethylase and *p*-nitroanisole-*O*-demethylase and the concentrations of cytochrome P450 and triglycerides. No other tissues were preserved and histopathological examination was not performed.

No deaths occurred during the study and clinical signs were confined to slight piloerection and ungroomed fur in the group treated at 0.235 mg/l, which persisted up to the fourth day of treatment. Clinical signs were not observed at lower exposure concentrations. There were no treatment-related effects at any exposure concentration on the neurological evaluation of reflexes. Body-weight gains, rectal temperatures and haematological profiles were unaffected by treatment at all exposure concentrations. Thromboplastin time was slightly, but significantly increased by 9% in male rats at 0.235 mg/l. Male rats of this group also showed slightly elevated plasma enzymes, which were statistically significant only for ALT (39%) and ALP (13%) activities. These effects were not also observed in females at 0.235 mg/l or in either sex at lower exposure concentrations. All other blood-chemistry parameters were unaffected by treatment.

All absolute and relative organ weights in the test groups were comparable to values for controls. No treatment-related gross lesions were observed at autopsy at any exposure concentration, after 7 or 21 days. Hepatic activities of the mixed function oxidase enzymes and concentrations of triglycerides were unaffected by treatment.

The NOAEL for repeated exposure to respirable prothioconazole-desthio by inhalation was 0.054 mg/l on the basis of increases in plasma ALT and ALP activities and a slight prolongation of thromboplastin time in male rats exposed at 0.235 mg/l (Pauluhn, 1991b).

Groups of 10 male and 10 female Wistar rats were exposed by inhalation to prothioconazole-desthio (purity, 95.4%) at analytically determined atmospheres of 0, 0.011, 0.047 or 0.228 mg/l in nose-only chambers for 6 h per day on five consecutive days per week for 4 weeks. The atmospheres were generated as an aerosol in 1 : 1 PEG 400/ethanol (0.20 ml/l, MMAD, ≤ 1.3 µm ± GSD 1.5 µm). There were two control groups, one that was exposed to 1 : 1 PEG 400/ethanol aerosol, and the other to air only. The actual exposure concentrations for the aerosols were measured analytically by HPLC in samples withdrawn from the breathing zone at the start, middle and end of each exposure period.

In addition to the standard observations, clinical examinations made on the 12th and 26th days of exposure included subjective evaluation of visual placing response, grip-strength on wire mesh, abdominal muscle tone, corneal and pupillary reflexes, pinnal reflex, righting reflex, tail-pinch response and startle reflex in five males and females per group. Rectal temperatures were recorded in five males and females per group before dosing and immediately after exposure on the 13th and 27th days. Ophthalmoscopic examinations were performed on five males and females per group before dosing and before termination. Blood chemistry analyses included T3 and T4 concentrations and measurement of TBC at autopsy. Additional samples of liver were retained for the determination of the tissue activities of aminopyrine-*N*-demethylase and *p*-nitroanisole-*O*-demethylase and tissue concentrations of cytochrome P450 and triglycerides. Additional liver samples were preserved and subsequently stained with oil red O, but no other histopathology was performed.

No deaths or clinical signs occurred at any exposure concentration. There were no effects on ophthalmological findings or on reflexes. Body-weight gains, rectal temperatures, haematological profiles and clinical chemistry profiles were unaffected by treatment at any concentration. Qualitative and semi-quantitative measurement of urinary constituents and cellular components did not show any treatment-related effects at any concentration.

There were no treatment-related effects at any concentration on absolute and relative organ weights or on the nature and incidence of gross lesions at autopsy. Statistically significant variations from the control values were observed in all of the biochemical measurements made on the liver, but the pattern of increases and decreases were not explicable in terms of a coherent response (Table 39).

The NOAEL for repeated exposure by inhalation to respirable prothioconazole-desthio was 0.228 mg/l (equivalent to 82.2 mg/kg bw per day) on the basis of no adverse effects at this concentration (Pauluhn, 1992).

Dogs

Groups of two male and two female beagle dogs were given diets containing prothioconazole-desthio (purity, 94.7%) at a concentration of 0, 10, 100, or 1000 ppm for 39 days. After 26 days, the dietary concentration of 100 ppm was increased to 5000 ppm for 13 days. Several other parameters in addition to those specified in the guidelines were measured: electrocardiographic (ECG) recordings, blood pressure and heart-rate measurements were performed before dosing and in week 4. In addition to the normal eye examinations, the pupillary, corneal, patellar, extensor, postural and flexor reflexes were evaluated before dosing and during week 4. Body temperature was recorded before dosing and during week 6. Haematology and blood chemistry analyses were performed on blood samples taken

Table 39. Liver enzyme activities and triglyceride concentration in rats exposed to prothioconazole-desthio by inhalation

Parameter	Exposure concentration (mg/l air [a])									
	Males					Females				
	0 (air only)	0 (vehicle only)	0.011	0.047	0.228	0 (air only)	0 (vehicle only)	0.011	0.047	0.228
O-Dem (mU/g)	10	8.9	7.3**	5.4**	10	7.9	8.7	6.9	7.0	9.4*
N-Dem (mU/g)	126	109*	101**	88**	143	65	69	59	64	75*
P450 (nmol/g)	36	35	33	36	41*	38	34	33**	33	39
Triglyceride (µmol/g)	5.4	5.4	6.3**	5.7	5.9	4.9	5.2	5.6*	5.6*	5.6

From Pauluhn (1992)

O-Dem, *p*-nitroanisole-*O*-demethylase; *N*-Dem, aminopyrine-*N*-demethylase.

* $p < 0.05$ (vs air control); ** $p < 0.01$ (vs air control).

[a] Particle-size analysis of the aerosol in the breathing zone showed all test groups to have a mass fraction in the respirable range (< 3 µm) of ≥ 97.9%.

before dosing and during weeks 2, 4 and 6. Additional liver samples were retained from all dogs for the determination of hepatic cytochrome P450, aminopyrine-*N*-demethylase, *p*-nitroanisole-O-demethylase, ECOD, EROD, ALD, EH, GST and GLU-T activities and triglyceride concentration. Sections of all preserved tissues from all dogs, including oil red O-stained freeze-sectioned liver, Prussian blue-stained spleen and PAS-stained kidney sections, were examined by light microscopy.

No deaths occurred during the study and there were no treatment-related clinical signs of a reaction to treatment at any dose. There were no treatment-related effects of food consumption or body-weight gain at doses up to 1000 ppm, but after the increase in dietary concentration from 100 to 5000 ppm in week 4, the dogs showed a marked decrease in food consumption and body-weight loss from 8.3 kg in week 4 to 7.6 kg in week 6. At these same times, the mean body weights of the controls were 8.2 kg and 8.5 kg, respectively.

No treatment-related effects were observed at any dose in the eye examinations, the neurological evaluation of reflexes or on body temperature. There were no treatment-related effects at any dose on heart rate or blood pressure, and the ECG traces showed no pharmacologically relevant changes.

No treatment-related effect was discernible from the haematology. The plasma ALP activity was increased in both males (20%) and females (50%) during the treatment period at 1000 ppm, and in males at 100/5000 ppm, compared with a progressive decline or no change in activity in the other treatment and control groups. All other blood-chemistry parameters at all doses were comparable to control values. Urine analysis did not show any treatment-related effects.

Other than thinness observed in three of the four dogs at 100/5000 ppm, there were no treatment-related gross pathology findings at autopsy; however, one female in the group at 1000 ppm group was pregnant. Liver weights were higher in the treated groups than in the controls, but with group sizes per sex of only two, statistical analysis was not appropriate.

Microscopic examination showed slight to moderate cytoplasmic changes in the liver, characterized by increased optical density and eosinophilic granulation of the hepatocyte cytoplasm, in the groups at 100/5000 and 1000 ppm. At 100/5000 ppm, the lesion was slightly more severe and covered the entire liver lobule whereas at 1000 ppm the changes were confined to the periphery of the lobule. Two of the four dogs in the group at 100/5000 ppm were found to have centrilobular hepatocytes that were slightly reduced in size. The hepatic effects at 100/5000 ppm are considered to be a consequence of glycogen

loss after body-weight loss. There were no effects at 10 ppm. Other histological changes noted in some dogs of some treated groups, but not in controls, were round cell infiltration of the adrenal glands, reduced cellularity of bone marrow and focal inflammation of the stomach.

The activities of the cytochrome P450-dependent monooxygenases, ECOD, ALD and EH and aminopyrine-*N*-demethylase, *p*-nitroanisole-*O*-demethylase and cytochrome P450 showed a marked treatment-related induction in the groups treated at 100/5000 and 1000 ppm. EROD activities were elevated in males only treated in the group at 100/5000 ppm. The phase II enzyme, GLU-T, also showed a marked induction in these groups. There was no effect on the activities of any of these enzymes in the group at 10 ppm. Triglyceride concentrations and GST activity were not affected by treatment at any dose.

The Meeting considered that the group sizes in this study were too small to permit a NOAEL to be identified with any confidence (Detzer & Rinke, 1999).

Groups of four male and four female beagle dogs were given diets containing prothioconazole-desthio (purity, 94.3%) at a concentration of 0, 40, 200, or 1000 ppm for 13 weeks. These dietary concentrations were equal to 0, 1.6, 7.8 and 37.8 mg/kg bw per day for males and 0, 1.6, 8.5 and 42.8 mg/kg bw per day for females. Several parameters in addition to those specified in the guidelines were measured. A number of measurements and observations were made before dosing and again in weeks 2, 6 and 13, these were: ECG recordings, blood pressure and heart rate measurements; eye examinations and, in addition, evaluation of the pupillary, corneal, patellar, extensor, postural and flexor reflexes; body temperature; haematology and blood-chemistry analyses, including serum T3 and T4 concentrations. Additional liver samples were retained from all dogs for the determination of hepatic cytochrome P450, aminopyrine-*N*-demethylase, *p*-nitroanisole-*O*-demethylase, ECOD, EROD, ALD, EH, GST and GLU-T activities and triglyceride concentration. Sections of all preserved tissues from all dogs, including oil red O-stained freeze-sectioned liver, Prussian blue-stained spleen and PAS-stained kidney sections, were examined by light microscopy.

No deaths occurred during the study and there were no treatment-related clinical signs of a reaction to treatment at any dose. There were no treatment-related effects of food consumption or body-weight gain at any dose.

There were no treatment-related effects at any dose on reflexes, body temperature, eye examinations, ECG, blood pressure or heart rate. Haematological profiles were unaffected by treatment at all doses. All plasma-enzyme, protein, substrate, electrolyte and hormone levels were unaffected by any treatment at any sampling time. Equally, there were no effects of treatment on urine analysis.

There were no treatment-related gross findings at necropsy. Treatment-related effects on organ weights were confined to a 20% increase in absolute and relative liver weights in females at 1000 ppm. The effect was not observed in males at 1000 ppm or in males or females at lower doses. All other organ weights were unaffected by treatment. Treatment-related histomorphological alterations were confined to the liver of dogs of males and females in the group at 1000 ppm. Three males and four females showed slight to moderate cytoplasmic ground-glass appearance, mainly of the centrilobular hepatocytes. This type of hepatic alteration is considered to indicate an increase in endoplasmic reticulum associated with enzyme induction.

The hepatic activities of aminopyrine-*N*-demethylase, *p*-nitroanisole-*O*-demethylase and cytochrome P450 were increased in some or all of the dogs at 1000 ppm. Liver triglyceride concentrations were increased in one male and one female at 1000 ppm. Hepatic ECOD activities were elevated by 151–171% in males and females at 1000 ppm, while EROD activities were unaffected by treatment at this dose. ALD and EH activities at 1000 ppm showed less marked increases of up to 78% in females, while males showed only slight increases of up to 46%. The activities of ECOD, EROD, ALD and EH in dogs at 40 and 200 ppm, and GST and GLU-T activities at all doses, were unaffected by treatment.

The NOAEL was 200 ppm, equivalent to 7.8 mg/kg bw per day, on the basis of increased liver enzyme activities, weights and histopathology at 1000 ppm, equal to 37.8 mg/kg bw per day (Hofmann et al., 2000).

Groups of four male and four female beagle dogs were given diets containing prothioconazole-desthio (purity, 92.8–93.1%) at a concentration of 0, 40, 300, or 2000 ppm for 30 weeks. These dietary concentrations were equal to 0, 1.4, 10.1 and 69.9 mg/kg bw per day for males and 0, 1.6, 11.1 and 77.1 mg/kg bw per day for females. The study had been designed as a 52-week study, but it was curtailed due to discontinuation of the development of this compound. A number of measurements and observations were made before dosing and again in weeks 5, 12 and 25. These were: ECG recordings, blood pressure and heart rate measurements; eye examinations and, in addition in week 1, evaluation of the pupillary, corneal, patellar, extensor, postural and flexor reflexes; body temperature; haematology and blood chemistry analyses, including serum T3 and T4 concentrations and T4 binding capacity. Additional liver samples were retained from all dogs for the determination of hepatic cytochrome P450, aminopyrine-*N*-demethylase, *p*-nitroanisole-*O*-demethylase and triglyceride concentration. Sections of all preserved tissues from all dogs were examined by light microscopy.

One male at 2000 ppm died on day 23. Microscopic examination of the dog showed it had severe focal infarction and congestion in the jejunum and blood in the colon. The cause of death was considered to be intestinal invagination. The dog that died was replaced and the new dog was treated for the remaining 178 days. No other premature deaths occurred and there were no treatment-related clinical signs of a reaction to treatment at any dose. There were no treatment-related effects on body weight or food consumption at any dose. There were no treatment-related effects at any dose in either sex on body temperature, reflex tests, blood pressure, heart rate, ECG, eye examinations, haematological profiles and urinary constituents.

Treatment-related effects on blood chemistry parameters were confined to the dogs treated at 2000 ppm. ALP activity was increased in males and females starting in week 5 and continuing to termination. At this same dose, T4 concentrations were decreased in males and females from week 5 and TBC was slightly increased in males and females in weeks 20 and 25. The effects were considered to be secondary to the enhanced metabolic activity of the liver after the induction of microsomal enzyme activity (see below). All other group mean data on blood chemistry in treated dogs were comparable to the control values.

There were no treatment-related gross findings during autopsy at any dose, apart from a marked increase in absolute and relative weights of the liver (25%) in males and females in the group at 2000 ppm.

Liver enzyme activities and triglyceride concentrations at autopsy in week 30 were elevated in males and females at 2000 ppm, but not at lower doses. At this dose, the increases in aminopyrine-*N*-demethylase and *p*-nitroanisole-*O*-demethylase activities and cytochrome P450 and triglyceride concentrations were 143%, 144%, 125% and 30%, respectively, in males and 287%, 135%, 167% and 36%, respectively, in females. Treatment-related microscopic findings were confined to the liver in males and females treated at 2000 ppm. The livers showed cytoplasmic changes in centrilobular hepatocytes in all dogs treated at 2000 ppm except for the one that died prematurely. The hepatocellular cytoplasm of the affected dogs was more homogeneous than usual, with a fine granular structure and reduction in glycogen storage. These findings usually represent an adaptive induction of hepatic enzymes, which would be consistent with the increased levels of hepatic enzymes and increased liver weight. There were no other treatment-related findings.

The NOAEL was 300 ppm, equal to 10.1 mg/kg bw per day, on the basis of changes in blood ALP activity and T4 and TBC and the occurrence of increased hepatic cytoplasmic changes at 2000 ppm equal to 69.9 mg/kg bw per day (Henninger et al., 2001).

4.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 60 male and 60 female B6C3F$_1$ mice were given diets containing prothioconazole-desthio (purity, 93.1 %) at a concentration of 0, 12.5, 50 or 200 ppm, with correction for purity, equal to 0, 3.1, 12.8 and 51.7 mg/kg bw per day for males and 0, 5.1, 20.3 and 80.0 mg/kg bw per day for females, for up to 105 weeks. Randomly selected groups of 10 males and 10 females were killed after 1 year of treatment. The doses for this study were selected on the basis of a 13-week study of dietary toxicity in mice (Wirnitzer, 1999, see above).

Survival at termination was satisfactory and mortality was not affected by treatment in either sex at any dose (Table 40). There were no treatment-related clinical signs, and food consumption and body-weight gain were unaffected by treatment. There were no consistent effects on haematological parameters over time, although there were occasional, small but statistically significant depressions (< 10%) in erythrocyte count, erythrocyte volume fraction and dependent parameters (haemoglobin concentration, MCV, MCH and mean corpuscular haemoglobin concentration (MCHC) at 200 ppm.

Triglyceride concentrations were statistically significantly reduced in week 105 in males at all doses (but not in females) and this may be related to altered liver function, however a dose–response was not evident. The reduction was also apparent in the groups dosed at 50 ppm and 200 ppm at week 53.

Creatinine concentrations were statistically significantly increased in males and females at 200 ppm in week 53, by slightly over 10%, although no effect was apparent at termination. Blood urea concentrations were statistically significantly reduced in males at all doses in week 105 and also in week 53 at 200 ppm, the greatest reductions reaching about 40%. No treatment-related histopathological changes in the kidneys of males or females at 200 ppm accompanied the observed higher plasma concentrations of creatinine, and the lower plasma concentrations of urea in all treated groups of males, which were probably not indicative of an adverse effect. There were no other treatment-related effects on clinical chemistry.

There were no treatment-related gross lesions at autopsy either of unscheduled decedents or in survivors killed after 1 or 2 years.

After 105 weeks, the absolute and relative liver weights of males and females at 200 ppm were increased by 12–17%. In addition, after 105 weeks, absolute and relative heart weights were reduced in male mice at 200 ppm and in female mice at 12.5, 50 and 200 ppm. No treatment-related histopathological changes in the hearts of male or female mice accompanied the reduced heart weights, which were probably not indicative of an adverse effect.

Treatment-related histopathological findings in mice killed after 1 year of treatment were confined to increased incidences of fine-vesicular vacuolation in the livers of male animals at 50 and 200 ppm. The incidences in oil red O-stained freeze-sectioned liver (Table 41) supported this observation. After 2 years of treatment, the incidences of periacinar fat accumulation in the liver were increased in males at 200 ppm and in females of all treated groups, although there was no statistical significance for the increased incidence in females at 12.5 ppm. There were no other treatment-related non-neoplastic findings after 1 or 2 years of treatment.

There was no evidence for a carcinogenic effect of prothioconazole-desthio at any dose.

The NOAEL was 12.5 ppm, equal to 3.1 mg/kg bw per day, on the basis of histopathological effects on the liver at 50 ppm, equal to 12.8 mg/kg bw per day. There was no evidence of a carcinogenic potential (Wirnitzer & Rinke, 2002).

Table 40. Non-histological effects in mice given diets containing prothioconazole-desthio for up to 2 years

Parameter	Week	Dietary concentration (ppm)							
		Males				Females			
		0	12.5	50	200	0	12.5	50	200
Mortality (%)	105	8	2	6	12	24	26	30	18
Blood chemistry									
Triglycerides (mmol/)	53	3.82	2.23	2.65*	1.98**	0.75	0.70	0.68	0.70
	105	1.63	0.96*	0.78**	0.79**	1.23	1.02	1.01	0.98
Creatinine (µmol/l)	53	25	26	26	28*	21	21	23	25**
	105	30	30	30	28	29	27	27	27
Urea (mmol/l)	53	14	13	13	9.7**	12	9.5**	11	10
	105	15	13**	12**	12**	13	13	12	11
Body and organ weights									
Body weight (g)	53	35	34	38	36	28	29	29	28
	105	35	34	34	33	31	29	30	30
Heart (mg)	53	194	194	185	201	162	161	164	160
	105	206	194	200	185**	180	169*	162**	165**
Relative weight of heart (mg/100g bw)	53	559	575	494	567	580	562	561	569
	105	599	577	592	559	591	574	545*	552*
Liver (g)	53	1.58	1.55	1.69	1.73	1.38	1.42	1.41	1.40
	105	1.84	1.78	1.87	2.06	1.58	1.48	1.62	1.76
Relative weight of liver (g/100g bw)	53	4.51	4.57	4.49	4.87	4.96	4.90	4.82	4.97
	105	5.34	5.27	5.51	6.23*	5.15	5.05	5.45	5.81

From Wirnitzer & Rinke (2002)

* $p < 0.05$; ** $p < 0.01$.

Rats

Groups of 50 male and 50 female Wistar rats (aged 6 weeks) were given diets containing prothioconazole-desthio (purity, 92.8–95.4 %) at a concentration of 0, 20, 140 or 980 ppm, with correction for purity, equal to 0, 1.1, 8.0 and 57.6 mg/kg bw per day for males and 0, 1.6, 11.2 and 77.4 mg/kg bw per day for females, for at least 106 weeks. Additional groups of 10 males and 10 females per group were treated similarly and were killed after at least 52 weeks of treatment.

Mortality was not affected by treatment (Table 42), and survival at termination was satisfactory. There were no treatment-related clinical signs and there were no effects on food consumption at any dose. Body-weight gain reductions of 13–16% in females at 980 ppm resulted in statistically significantly lower body weights after 52 and 103 weeks of treatment of 5% and 7%, respectively. There were no significant effects on the body weights of male rats.

Ophthalmoscopy showed no treatment-related ocular changes in rats at 980 ppm. A treatment-related minimal and transient effect on erythrocyte parameters occurred at 980 ppm. Haemoglobin concentrations and values for erythrocyte volume fraction in males and females and erythrocyte counts in females were statistically significantly lower by 10% or less in weeks 28 and 53, but not at subsequent sampling times. In males only, MCHC values were statistically significantly lower at 980 ppm at all sampling times, but the reductions were never more than 3% and the effect was not

Table 41. Non-neoplastic histological effects in mice given diets containing prothioconazole-desthio for up to 2 years

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	12.5	50	200	0	12.5	50	200
1 year (including unscheduled deaths)								
Liver, No. examined	10	10	13	10	10	10	10	10
Hepatocellular vacuolation	1	1	3	6	0	0	1	0
Periacinar fat staining/	1	2	7	8	2	4	3	3
Cytoplasmic change of hepatocytes (hypertrophy)	0	0	0	1	0	0	3	5
2 years								
Liver , No. examined	44	49	47	44	38	37	35	41
Periacinar fat staining[a]								
Minimal	0	5	0	0	1	0	0	0
Slight	15	11	21	17	7	14	15	10
Moderate	2	0	1	12	0	2	8	14
Total	17	16	22	29	8	16	23	24
2 years (including unscheduled deaths)								
Liver, No. examined	50	50	50	50	49	50	50	50
Periacinar fat staining[a]	20	17	22	32*	11	21	28***	25**

From Wirnitzer & Rinke (2002)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Fisher's exact test).

[a] Indicative of fine-vesicular vacuolation.

Table 42. Non-histological effects in rats given diets containing prothioconazole-desthio for up to 2 years

Parameter	Week	Dietary concentration (ppm)							
		Males				Females			
		0	20	140	980	0	20	140	980
Mortality (%)	106	12	14	28	12	32	36	28	16
Body weight (g)	0	119	116	118	119	103	105	105	106*
	53	439	428	434	430	246	249	240	234*
	103	438	436	437	430	272	273	269	252**
Body-weight gain (g)	0–53	269	269	268	261	139	137	129	121
	53–103	268	276	270	261	164	160	156	138
Haematology									
Erythrocytes (10^{12}/l)	28	9.36	9.20	9.12	9.04	8.62	8.35	8.42	7.92**
	53	9.22	9.14	9.08	9.29	8.54	8.16	8.44	7.96**±
Haemoglobin (g/l)	28	156	152	151	144**	152	147	147*	143**
	53	159	153	155	152	156	148**	153	149**
Haematocrit[a] (%)	28	47.4	46.7	46.4	45.3*	46.3	45.1	45.2	43.4**
	53	47.1	45.7	46.3	45.8	45.4	43.4*	44.9	43.1**

MCHC (g/l of erythrocytes)	28	327	325	325	318**	328	326	325	329
	53	337	335	334	330*	345	342	341	345
	79	326	324	322	319*	335	330	327	326
	104	328	323	323	320*	326	324	328	324
Leukocyte count (10^9/l)	28	9.0	8.4	8.4	8.6	7.3	6.2	5.4*	5.7
	53	7.3	7.5	8.3	7.2	5.9	6.0	5.6	4.7*
	79	6.3	7.6	7.0	7.2	6.2	7.3	6.0	4.8
	104	7.9	7.3	7.6	5.5*	6.6	6.8	5.1	4.2*
Blood chemistry									
AST (U/l)	28	31	31	31	31	40	38	31	32*
	53	43	34	42	38	60	64	40*	37*
	79	40	31	33	31	48	58	41	40
	104	34	31	30	30	48	43	38	40
ALP (U/l)	28	184	188	172	160	142	122	124	113*
	53	177	192	190	172	121	118	118	105
	79	178	196	180	166	146	121	126	122
	104	180	185	161	159	164	151	133	139
GLDH (U/l)	28	6.2	3.6	4.3	2.3	15	12	5.6	8.5
	53	26	12	19	8.4	73	56	28	17
	79	27	13	13	6.8	30	29	20	12
	104	17	10	5.9*	4.0*	29	15	7.8	2.9
T3 (nmol/l)	28	1.43	1.77*	1.70*	1.54	1.42	1.45	1.45	1.55
	53	1.45	1.74**	1.50	1.57	1.66	1.61	1.57	1.41*
	79	1.53	1.51	1.47	1.45	1.43	1.40	1.41	1.38
	104	1.46	1.42	1.45	1.32	1.46	1.52	1.34	1.30*
T4 (nmol/l)	104	54	50	42*	40*	40	42	36	39
Bilirubin (µmol/l)	28	1.6	1.7	1.6	1.3**	1.5	1.3	1.3	1.1**
	53	1.1	1.3	1.2	0.9	1.5	1.4	1.4	1.0**
	79	1.6	1.6	1.5	1.2*	1.6	1.6	1.3	1.3
	104	2.2	2.2	1.8	1.5**	1.9	1.8	1.6	1.6*
Organ weights									
Body weight (g)	52	437	445	432	430	255	242	251	232
	104	442	437	436	428	273	272	270	253**
Liver (g)	52	15	15	15	18	9.3	8.2	8.8	9.5
	104	16	15	15	17**	10	10	10	11
Relative weight of liver (g/100 g bw)	52	3.4	3.3	3.4	4.2**	3.7	3.4	3.5	4.1*
	104	3.5	3.4	3.5	4.0**	3.7	3.7	3.7	4.3**
Ovaries (mg)	52	—	—	—	—	128	147	147	140
	104	—	—	—	—	135	161	143	162
Relative weight of ovaries (g/100 g bw)	52	—	—	—	—	50	61	59	61
	104	—	—	—	—	50	58	53	64**

From Schladt et al. (1999)

* $p < 0.05$; ** $p < 0.01$.

[a] Haematocrit is equivalent to erythrocyte volume fraction/100.

progressive. At 980 ppm, leukocyte counts were statistically significantly lower by 20% in females in week 53, and in males and females at week 104, by 30% and 36%, respectively; however, differential leukocyte counts were not affected at any dose.

The activity of plasma liver enzymes, AST and ALP, was significantly depressed by 20% in each case in females of the group at 980 ppm at week 28, and AST was also reduced in females of the groups at 140 ppm and 980 ppm at week 53, by 33% and 38%, respectively. No significant reductions in enzyme activity were observed at later sampling times, although the activities were lower in the groups at the intermediate and highest dose compared with the controls. The plasma activity of GLDH was statistically significantly reduced in males of the groups at 140 ppm and 980 ppm only at termination, by 65% and 76%, respectively. Total plasma concentrations of bilirubin were significantly reduced by between 16% and 33% at 980 ppm in male and female rats at most sampling times. T4 concentrations were statistically significantly reduced in males at 140 and 980 ppm in week 104, by 22% and 26%, respectively. There was no change in T4 concentration in female rats. T3 concentrations were reduced in females at 980 ppm, at weeks 53 and 104, but not at weeks 28 or 79. In males, T3 was significantly increased in some groups at weeks 28 (20 ppm and 140 ppm) and 53 (20 ppm), but the lack of any dose–response relationship points to these variations being random fluctuations rather than an effect of treatment. No other blood-chemistry parameters evaluated were affected by treatment. Urine analysis did not show any effects of treatment.

Among the rats killed after 1 year, pale-coloured livers were observed in 2 out of 10 and 9 out of 10 males at 140 and 980 ppm, respectively. At 2 years, females in the group at 980 ppm showed higher incidences of cysts in the liver (eight cases vs one case in the control group). In the group at 980 ppm, there were increases in liver weights relative to body weight of 24% and 11% in males and females, respectively, after treatment for 1 year and of 14% and 16% in males and females, respectively, after 2 years. The absolute weight of the liver was also increased by 6% in male rats of the group at 980 ppm after 2 years of treatment. The ovary weights relative to body weight were significantly increased by 28% after 2 years of treatment at 980 ppm.

Notable observations made upon microscopic examination (Table 43) after 1 year of treatment were hepatocellular vacuolation in all 10 male rats in the group at 980 ppm and cytoplasmic change in 8 out of 10 male rats, compared with none in the controls, for either lesion. Periportal fatty change was observed in several groups of males and females, while centrilobular fatty changes was only observed in 3 out of 10 females of the group at 980 ppm. Single-cell necrosis was observed in 2 out of 10 males of the group at 980 ppm, but neither males nor females of any other group.

The incidences of hepatic lesions became statistically significantly higher after 2 years of treatment. This was particularly so for hepatocellular vacuolations due to lipid accumulation, single cell fatty change and centrilobular fatty change in males at both 140 ppm and 980 ppm. In addition, increased incidence of hepatocellular hypertrophy and cytoplasmic change were observed in male rats at 980 ppm. Female rats appeared to be less affected at 2 years. Nevertheless, statistically significant increases were observed in the incidence of single-cell fatty change at 140 ppm and 980 ppm.

At 2 years, there was a low but statistically significantly increased incidence of C-cell hyperplasia in the thyroid of male rats at 980 ppm. In female rats, the incidences of colloidal mineralization fluctuated over the dose range. In males, the incidences of colloidal mineralization tended to be high at all doses. Other non-neoplastic microscopic observations of note made at 2 years included increased incidences of adrenal cortical vacuolation in males at 140 and 980 ppm and focal adrenocortical hyperplasia in females at 980 ppm, with increased incidence of ovarian follicular cysts. Females with corpora lutea in the ovaries were seen more frequently in the groups at 140 ppm and 980 ppm. Atrophy of this organ was correspondingly reduced in these groups. In lungs of females, foam-cell accumulation was significantly increased at 980 ppm.

With few exceptions, the majority of tumours were evenly distributed among the groups. The only tumour type for which there was a significant increase was Leydig-cell adenomas at 140 ppm.

Table 43. Non-neoplastic histological effects in rats given diets containing prothioconazole-desthio for up to 2 years

Effect	Dietary concentration (ppm)							
	Male				Female			
	0	20	140	980	0	20	140	980
1 year								
Liver, No. examined	10	10	10	10	10	10	10	10
Hepatocellular vacuolation	0	0	3	10	0	0	0	3
Single-cell necrosis	0	0	0	2	0	0	0	0
Fatty change (centrilobular)	0	0	0	0	0	0	0	3
Fatty change (periportal)	3	4	8	1	1	0	2	5
Cytoplasmic change	0	0	1	8	0	0	0	0
Thyroid, No. examined	10	10	10	10	10	10	10	10
Follicular epithelial hypertrophy	0	1	1	3	0	0	0	0
Colloidal mineralization	3	2	2	5	1	0	1	3
2 years								
Liver, No. examined	50	50	50	50	50	50	50	50
Hepatocellular vacuolation	2 t**	6	26**	42**	16 t**	15	23	39**
Fatty change (single cell)	6 t*	6	15*	18**	15 t**	15	29**	25*
Fatty change (centrilobular)	0 t**	1	7**	30**	3	0	1	1
Fatty change (periportal)	29	24	23	13	13	13	16	19
Pigment-laden Kupffer cells	1 t**	0	1	1	9 t**	10	15	15
Hepatocellular hypertrophy	1 t**	0	2	24**	0 t**	2	2	18**
Cytoplasmic change	1	1	2	40**	11 t**	8	17	39**
Lung, No. examined	50	50	50	50	50	50	50	50
Foam-cell accumulation	5	7	4	5	3 t*	7	5	12**
Ovaries, No. examined	—	—	—	—	50	50	50	50
Follicular cysts	—	—	—	—	12 t**	7	16	23*
Corpora lutea	—	—	—	—	20 t**	12	31*	31*
Atrophy	—	—	—	—	25	28	17	15
Thyroid, No. examined	50	50	50	50	49	50	50	50
C-cell hyperplasia/focal	2 t*	3	5	7*	6	9	8	10
Colloidal mineralization	39	29	36	41	15 t**	24*	14	35**
Adrenal gland, No. examined	50	50	49	50	50	50	50	50
Vacuolation (cortical)	12 t*	11	20*	20*	3	1	0	1
Focal adrenocortical hyperplasia	1	1	1	0	3 t*	3	4	10*

From Schladt et al. (1999)

t* $p < 0.05$; t** $p < 0.01$ (trend test); * $p < 0.05$; ** $p < 0.01$ (pair-wise group comparison).

The incidence was 14% compared with 4% in the controls and 4% at 980 ppm. The higher incidence at 140 ppm remained well within the range for historical controls (2–22%) and the lack of a dose-related response also suggested that the observation was of no biological significance. Decreased incidences of some tumour types were statistically significant in some cases: in males, these were granular cell tumours in the brain, liposarcomas in the kidneys and medullary tumours in the adrenals;

in females, these were pars distalis adenomas of the pituitary, tumours of the mammary glands and thymic thymomas. The Meeting did not suggest that treatment with prothioconazole-desthio had any protective effect.

The NOAEL was 20 ppm, equal to 1.1 mg/kg bw per day, on the basis of effects on liver and ovaries at 140 ppm, equal to 8.0 mg/kg bw per day. There was no evidence of carcinogenic potential (Schladt et al., 1999).

4.4 Genotoxicity

Prothioconazole was tested for genotoxicity in a range of assays, both in vitro and in vivo (Table 44). There was no evidence for induction of gene mutation in any of these assays.

4.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a pilot study to determine doses for a multigeneration study, groups of 10 male and 10 female Sprague-Dawley rats were given diets containing prothioconazole-desthio (purity, 95.4–95.6%) at a concentration of 0, 10, 50, 1000 or 1500 ppm. Treatment started 4 weeks before mating and continued throughout gestation and lactation until postnatal day 21.

The NOAEL for reproductive effects was 50 ppm on the basis of decreased litter size at birth, reduced neonatal viability, growth retardation before weaning and liver discoloration in the offspring at 1000 ppm. In addition, treatment at 1500 ppm resulted in a decrease in the number of pregnant females producing live-born pups (gestation index) and cleft palate in a small number of offspring. The NOEL for all effects in male and female parental rats was 50 and 10 ppm, respectively, on the basis of the occurrence of increased liver weight in males at 1000 ppm and in females at 50 ppm (Eigenberg & Hoss, 1992).

Table 44. Results of studies of genotoxicity with prothioconazole-desthio

End-point	Test object	Concentration/dose[a] (LED/HID)	Purity (%)	Result	Reference
In vitro					
Gene mutation	*S. typhimurium* strains TA100, TA1535, TA1537, TA98; standard plate test and pre-incubation	600 µg/plate ± S9	93.7	Negative	Herbold (1990)
Gene mutation	Chinese hamster lung V79d cells, *Hprt* locus	500 µg/ml	92.7–93.1	Negative	Brendler-Schwaab (1999b)
Chromosomal aberration	Chinese hamster ovary CHO cells	125 µg/ml	93.1	Negative	Gahlmann (1995)
Unscheduled DNA synthesis	Rat hepatocytes	60 µg/ml	93.7	Negative	Brendler (1992)
In vivo					
Micronucleus formation	Male and female NMRI mice, bone-marrow cells	350 mg/kg bw × 1, intraperitoneal, 16, 24 and 48 h after dosing	93.1	Negative	Herbold (1993)

LED, lowest effective dose; HID, highest ineffective dose; S9, 9000 × *g* supernatant from livers of male rats.

Groups of 30 male and 30 female Sprague-Dawley-derived rats were given diets containing technical-grade prothioconazole-desthio (purity, 92.8–95.6% in four analyses) at a concentration of 0, 40, 160 or 640 ppm with 1% corn oil. This dose range was selected on the basis of the results of a preliminary study (Eigenberg & Hoss, 1992). The actual doses received are given in Table 45.

Exposure was continuous from 10 weeks before mating until to weaning of F_1 generation pups. F_1 progeny were then treated similarly until weaning of the F_2 generation pups. Observations included body weights, food consumption (not during mating period), clinical signs and litter parameters. The estrous cycle was assessed over a 2 week pre-mating period in 10 females per group (parental F_{ti} and F_1 generations). Day of vaginal opening and preputial separation were recorded in F_1 rats. Anogenital distance was measured on the day of birth. All rats were subjected to autopsy at termination and selected organs weighed. Reproductive organs and tissues, pituitary gland, liver and gross lesions were preserved from all parental animals of both generations and examined microscopically. A quantitative evaluation of the ovaries for pre-antral follicles, antral follicles and corpora lutea was performed for all females in the control groups, and for females of the F_0 and F_1 generations receiving prothiocoazole-desthio at 160 and 640 ppm.

In the F_0 generation, one female in the control group and one female at 640 ppm died showing evidence of dystocia (difficulty in giving birth), and a further three females at 640 ppm showing dystocia were killed in a moribund condition. In the F_1 generation, three females at 640 ppm showing dystocia were killed in a moribund condition. There were no treatment-related clinical signs in parental animals of either generation at any dose.

There were no treatment-related effects on the body-weight gain of parental animals of either generation at any dose. Similarly, there were no treatment-related effects on the food consumption of males at any time and of females during the pre-mating period and gestation. However, females of F_0 and F_1 generations treated at 640 ppm showed a treatment-related decrease of 8–17% in food consumption during lactation. This effect was considered to be a reflection of the smaller litters and pup weights at 640 ppm. Food consumption of rats at lower doses was unaffected by treatment.

There were no effects on the characteristics of the estrous cycle of either generation at any dose. The group mean frequency of estrous cycles was 2 per 14 days for all treated and control groups of both generations. There were no treatment-related effects on the mating, fertility, gestation and birth indices, the time to insemination, the duration of gestation and the mean number of implantation sites in either generation at any dose (Table 46). Although the fertility indices for both generations treated at 640 ppm were lower than the indices for the control groups, this was not statistically significant and appeared to be part of normal variation when compared with the range of indices in other groups of both generations. The significantly lower time to insemination recorded for the F_1 generation treated at 640 ppm was considered to be incidental to treatment with prothioconazole-desthio. The frequencies of dystocia in the F_0 and F_1 generations at 640 ppm were elevated by about

Table 45. Actual doses received in a multigeneration study in rats given diets containing prothioconazole-desthio

Period		Actual dose (mg/kg bw per day)		
		Nominal dietary concentration		
		40 ppm	160 ppm	640 ppm
Pre-mating	Males	2.7	10.4	42.6
	Females	3.0	12.0	49.5
Gestation		2.5	10.0	41.2
Lactation (days 0–14)		4.8	18.6	72.6

From Eigenberg & Lake (2001)

10% in comparison with the concurrent control group and the database of historical controls for the laboratory (range, 0–4%). Although these frequencies were not statistically significantly increased, the Meeting considered them to be related to treatment.

The only treatment-related clinical sign observed in the progeny of the F_1 and F_2 generations was an increased incidence of cannibalized (or missing) pups at 640 ppm. The number of litters with cannibalized pups was statistically significantly elevated in the F_1 generation (11 out of 22 compared with 4 out of 27 controls) and the F_2 generation (10 out of 17 compared with 3 out of 21 controls). There were no treatment-related effects on litter size, sex ratio at birth, live birth index or lactation index (Table 47). Although the mean size of F_1 generation litters at 640 ppm was statistically significantly lower than the vaue for the controls, the Meeting considered that this was an effect

Table 46. Summary of reproductive data from a multigeneration study in rats given diets containing prothioconazole-desthio

	Dietary concentration (ppm)			
	0	40	160	640
F_0 generation				
No. of females paired	30	30	30	30
No. of females with implantation sites	29	25	24	23
No. of females with live-born pups	27	23	24	22
Mating index [a] (%)	100	93	100	97
Fertility index [b] (%)	97	89	80	79
Gestation index [c] (%)	93	92	100	96
Time to insemination (days)	3.7	5.5	3.2	4.3
Duration of gestation (days)	21.8	22.1	22.0	22.1
Birth index[d,f] (%)	87	82	87	82
Mean No. of implantations [f]	18	16	17	17
F_1 generation				
No. of females paired	30	30	30	30
No. of females with implantation sites	21	23	22	17
No. of females with live-born pups	21	22	21	16[e]
Mating index [a] (%)	97	90	100	90
Fertility index [b] (%)	72	85	73	63
Gestation index [c] (%)	100	96	95	100
Time to insemination (days)	5.8	6.2	5.2	3.2*
Duration of gestation (days)	22.0	22.3	22.1	22.3
Birth index[d,f] (%)	83	82	80	81
Mean No. of implantations [f]	17	16	15	15

From Eigenberg & Lake (2001)

[a] Mating index = No. of inseminated females/No. females co-housed with male × 100

[b] Fertility index = No. of pregnant females/No. sperm-positive females × 100

[c] Gestation index = No. of females with live pups at birth/No. pregnant females × 100

[d] Birth index = No. of pups born per litter/implantation sites per dam × 100

[e] One female delivering one pup was excluded.

[b] Excludes pups from dams with dystocia.

*$p < 0.05$.

of treatment with prothioconazole-desthio since a mean litter size of 13 falls within the range for historical controls for the laboratory (range, 11–16; 23 studies performed between 1989 and 1997) and a similar effect was not observed in the F_2 generation. There was, however, a treatment-related decrease in the viability index at 640 ppm that was statistically significant in both the F_0 and F_1

Table 47. Summary of litter data in a mutigeneration study in rats fed diets containing prothioconazole-desthio

Litter data	Dietary concentration (ppm)			
	0	40	160	640
F_0 generation				
No. of litters	27	23	24	22
Mean litter number at birth[a]	16	16	14	13*
No. of stillborn pups	1	0	1	1
Mean pup weight (g) [a]:				
Day 0	6.6	7.0*	6.9	6.5
Day 4[b]	10.2	10.5	10.3	9.2
Day 7	16.8	17.4	16.7	14.3*
Day 14	33.4	31.6	33.0	29.9*
Day 21	51.7	51.2	51.3	46.4*
Mean no. of viable pups on day 21	8	8	8	7
Live birth index (%)[c]	97	98	96	95
Viability index (%)[d]	98	99	99	77*
Lactation index (%)[e]	100	99	100	94
Cannibalized pups	6	6	2	35
F_1 generation				
No. of litters	21	22	21	17
Mean litter No. at birth[a]	14	14	14	13
No. of stillborn pups	0	0	1	1
Mean pup weight (g) [a]				
Day 0	6.7	7.2	7.1	6.6
Day 4	10.2	11.2	11.1	9.7
Day 7	16.6	18.2	17.6	14.8
Day 14	32.5	35.8*	33.5	28.7*
Day 21	50.9	55.7*	52.7	46.6*
Mean No. of viable pups on day 21	8	8	8	6
Live birth index (%)[c]	99	98	92	94
Viability index (%)[d]	98	99	99	66*
Lactation index (%)[e]	99	99	99	95
Cannibalized pups	4	3	6	29

From Eigenberg & Lake (2001)

[a] Excludes data from dams with dystocia.

[b] Pre-cull or post-cull not specified.

[c] Live birth index = No. of live births per litter/total No.of pups per litter × 100

[d] Viability index = No. of live pups per litter on day 4/No. of live births per litter × 100

[e] Lactation index = No. of live pups per litter on day 21/No.of live pups per litter on day 4 (post-cull) × 100

* $p < 0.05$

generations, indicating decreased neonatal viability. The effect was not apparent at lower doses. Mean birth weights were unaffected by treatment at all doses in both generations. However, statistically significant growth retardation was observed subsequently in both generations at 640 ppm. Pup-weight gain during lactation was unaffected at lower doses.

Other than the treatment-related dystocia at 640 ppm, there were no treatment-related gross lesions or effects in adult males or females in either generation. In the F_1 generation there were three pups in two litters of the group at 640 ppm that had cleft palate. On the basis of the low spontaneous incidence of cleft palate in the strain of rat used, this finding was considered to be a treatment-related effect. No treatment-related gross lesions occurred in pups of either generation killed at age 21 days.

There were increases in absolute and relative liver weights in males of the F_0 generation and males and females of the F_1 generation in the group at 640 ppm; some of these increases were statistically significant (Table 48). Upon microscopic examination of the livers, increases were observed in the incidence and severity of multifocal hepatocellular cytoplasmic vacuolation in male rats of the groups at 160 ppm and 640 ppm in the F_0 and F_1 generations. The incidence of multifocal hepatocellular cyotoplasmic vacuolation was also observed, but with relatively low severity scores, in females of the group at 640 ppm in both the F_0 and F_1 generations. Hepatic necrosis was observed at increased incidence in females of the group at 640 ppm of both the F_0 and F_1 generations. Since many of the females at 640 ppm that showed dystocia also exhibited moderate hepatic necrosis, the former may be secondary to hepatic necrosis (Table 49).

The absolute and relative ovary weights of females of the F_1 generation at 640 ppm and the absolute weight at 160 ppm were statistically significantly higher than the values for controls. These changes were not considered to be compound-related since there was no dose-related increase in the F_1 generation, the F_0 generation was not affected, the increase was minimal, and the increase was not associated with the observation of histological lesions or functional changes in reproductive parameters. The mean numbers of antral follicles in the ovaries of females treated at 640 ppm of both generations were higher than the control values and statistically significantly increased in the F_1 generation (Table 50). However, the mean numbers of pre-antral follicles and corpora lutea were not significantly different from the controls and consequently the difference in antral follicle counts at 640 ppm was considered to be incidental to treatment with prothioconazole-desthio (Eigenberg & Lake, 2001).

Table 48. Organ-weights of parental animals in a multigeneration study in rats fed diets containing prothioconazole-desthio

Organ	Dietary concentration (ppm)							
	Males				Females			
	0	40	160	640	0	40	160	640
F_0 generation								
Absolute liver weight (g)	27	28	28	32*	21	21	22	23
Relative liver weight (% bw)	4.4	4.5	4.5	5.2*	5.6	5.7	6.0	6.3
Absolute ovary weight (mg)	—	—	—	—	170	210	182	189
Relative ovary weight (% bw)	—	—	—	—	0.046	0.060	0.049	0.052
F_1 generation								
Absolute liver weight (g)	30	29	30	31	19	21	22	22
Relative liver weight (% bw)	4.6	4.4	4.5	4.9*	5.3	5.6	5.8	6.2*
Absolute ovary weight (mg)	—	—	—	—	171	174	206*	201*
Relative ovary weight (% bw)	—	—	—	—	0.047	0.046	0.056	0.058*

From Eigenberg & Lake (2001)

* $p < 0.05$

Table 49. Treatment-related histopathological changes to the liver in a multigeneration study in rats fed diets containing prothioconazole-desthio

Histopathological change	Dietary concentration (ppm)							
	Males				Females			
	0	40	160	640	0	40	160	640
F_0 generation								
Liver, No. examined	30	30	30	30	30	30	30	30
Vacuolation of hepatocytes	13	20	23*	29*	0	0	0	9*
Average severity grade	1.4	1.5	2.0	2.8	—	—	—	1.4
Necrosis	0	0	1	0	1	0	1	4
Average severity grade	—	—	3.0	—	2.0	—	2.0	2.8
F_1 generation								
Liver, No. examined	30	30	30	30	30	30	30	30
Vacuolation of hepatocytes	14	16	26*	30*	0	5*	2	8*
Average severity grade	1.7	1.4	1.7	2.8	—	1.6	1.5	1.6
Necrosis	0	0	0	1	0	0	1	4
Average severity grade	—	—	—	3.0	—	—	2.0	2.5

From Eigenberg & Lake (2001)

* $p < 0.05$

Table 50. Group mean counts of ovarian follicles and corpora lutea in a multigeneration study in rats fed diets containing prothioconazole-desthio

Count	Dietary concentration (ppm)			
	0	40	160	640
F_0 generation				
Pre-antral follicle	18.97	—	18.87	19.80
Antral	17.87	—	18.07	21.37
Corpora lutea	15.47	—	14.57	15.47
F_1 generation				
Pre-antral	17.00	—	14.03	15.00
Antral	16.30	—	15.77	19.40*
Corpora lutea	15.20	—	15.37	14.87

From Eigenberg & Lake (2001)

— Not examined.

* $p < 0.05$

The NOAEL for parental effects was 40 ppm or 160 ppm, equal to 2.7 or 10 mg/kg bw per day in males or female rats, respectively, on the basis of hepatocellular vacuolation in males at 160 ppm equal to 10.4 mg/kg bw per day and on the basis of mortality (due to dystocia), decreased food consumption, hepatocellular vacuolation and necrosis (especially in individuals with dystocia) in females at 640 ppm equal to 41.2 mg/kg bw per day. The NOAEL for reproductive effects was 640 ppm, equal to 42.6 mg/kg bw per day, the highest dose tested, in males, and 160 ppm, equal to 10.0 mg/kg bw per day in females, on the basis of the occurrence of dystocia at 640 ppm, equal to 41.2 mg/kg bw per day. The NOAEL for effects upon the offspring was 160 ppm, equal to 10.0 mg/

kg bw per day, on the basis of the occurrence of decreased neonatal viability, pre-weaning growth retardation and an increased incidence of cleft palate at 640 ppm, equal to 41.2 mg/kg bw per day (Eigenberg & Lake, 2001).

(b) Developmental toxicity

Rats

A non-GLP dose range-finding study was performed to determine suitable doses for studies of developmental toxicity in rats. Two groups of 25 mated Wistar rats were given prothioconazole-desthio (purity, 99.1%) at a dose of 0 and 100 mg/kg bw per day by gavage in 0.5% aqueous Cremophor EL from day 6 to day 15 post coitum. On day 20 of gestation, the females were killed and assessed by gross pathology. The maternal rats were examined for general tolerance of prothioconazole and intrauterine development (implantations and resorptions). Body weights, food and water consumption were recorded. The fetuses were examined for external and skeletal abnormalities.

There were no deaths or treatment-related clinical signs during the course of the study. The food consumption and body-weight gains of the rats at 100 mg/kg bw per day were unaffected by treatment. There were no treatment-related gross lesions at autopsy of the females. All reproductive parameters were comparable to those of the controls with the exception of mean placental weight, which was statistically significantly higher in rats at 100 mg/kg bw per day. There were treatment-related increases in the incidences of fetuses with minor skeletal variants and with malformations (limb dysplasia, macroglossia, cleft palate), which were markedly and statistically significantly increased at 100 mg/kg bw per day. The increased incidence of retarded skeletal ossification is indicative of developmental toxicity and the increased incidence of malformations is indicative of a teratogenic response.

The NOAEL for maternal effects was 100 mg/kg bw per day, the only dose tested. This dose was developmentally toxic and teratogenic (Renhof, 1990).

In a study of developmental toxicity, groups of 35 time-mated, female Wistar rats were given prothioconazole-desthio technical (purity, 97.4%) at a dose of 0, 10, 30 or 100 mg/kg bw per day by gavage as a suspension in 0.5% aqueous Cremophor EL on days 6 to 15 post coitum. The day of confirmation of mating (when spermatozoa were detected) was designated day 0 of gestation. Groups of 10 rats at each dose were killed and examined on day 16 of gestation. On day 21 of gestation, the remaining 25 females per group were killed and assessed for gross pathology. Liver weights were recorded and histopathological examinations were performed on liver from all dams. Blood were taken on day 16 for the determinations of AST and ALT activity. 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 stained for skeletal examination, while the others were examined for soft tissue alterations.

No deaths occurred during the study and there were no treatment-related clinical signs at any dose. Food consumption and body-weight gain of the group at 100 mg/kg bw per day were 8% and 11% lower, respectively, than values for the control group. The body-weight gain and food consumption of other treated groups were unaffected by treatment. There were no treatment-related findings at autopsy.

The incidence of pregnancy, mean numbers of corpora lutea and implantations per dam and preimplantation losses were comparable in all treated and control groups. However, at doses of 30 and 100 mg/kg bw per day there were statistically significant increases in the percentage of implantations that were embryonic resorptions of 7.5% and 14.7%, respectively compared with 3.6% in the control group. In addition, at 100 mg/kg bw per day there was a statistically significant increase in

the percentage of implantations that were fetal deaths of 4.4%, compared with 0.3% in the control group. Although high, the percentage of embryonic resorptions in the group at 30 mg/kg bw per day lay within the range of variation among historical controls for this strain of rat (2.8–8.4% in 24 studies carried out between March 1986 and January 1989). The sex ratios and mean pup weights were unaffected by treatment at all doses.

The occurrence of palatoschisis (cleft palate) in two fetuses from different dams treated at 100 mg/kg bw per day, compared with a single incidence of this abnormality in the data on historical controls for the laboratory, suggested that this effect may be related to treatment. There were also statistically significant increases in the litter- and fetus-based incidences of single unilateral or bilateral supernumerary ribs (no differentiation made between rudimentary – a common spontaneous variation – and fully-formed supernumerary ribs – a malformation – at all doses of prothioconazole-desthio (Table 51). In addition, there were statistically significantly increased incidences of absent or incomplete ossification of one or more sternebrae, the first cervical vertebra, and of unossified limb phalanges in the group treated at 100 mg/kg bw per day and some of these effects were observed at 30 mg/kg bw per day. The os occipitale was incompletely ossified in all treated groups. Delayed ossification was not associated with any effects on fetal weights. The incidences of fetuses with a supernumerary rib were statistically significantly increased at all doses.

In the subgroups killed on day 16 of gestation, there was no effect of treatment on the rate of pregnancy. There were no treatment-related gross findings at autopsy, but the level of postimplantation losses (12%) in rats at 100 mg/kg bw per day was increased above the range for historical controls. The plasma activities of AST and ALT were unaffected by treatment, while there were statistically significant increases of 14–19% in the absolute and relative weights of the liver in rats at 100 mg/kg bw per day. Inflammatory foci in the liver occurred in all except one rat in the control group, but the severity was increased from a mean score of 1.6 in the control group to 2.1 in rats at 100 mg/kg bw per day. Treatment at 100 mg/kg bw per day also elicited minimal to moderate centrilobular hypertrophy and both centrilobular and periportal fatty change.

The cleft palates seen in the main group occurred only at the highest dose, while supernumerary ribs occurred at all doses, indicating that the teratological effects seen may not be have been related to effects in the liver.

The NOAEL for maternal rats was 30 mg/kg bw per day on the basis of reduced food consumption and body-weight gain, increased liver weight and histological changes in the liver at 100 mg/kg bw per day. No NOAEL for developmental toxicity was identified since an increased incidence of supernumerary ribs (no differentiation made between rudimentary and fully-formed) and an incomplete ossification of os occipitale occurred at all doses tested. Treatment at 100 mg/kg bw per day also elicited increased palatoschisis, embryonic (or early) and fetal (or late) deaths, decreased live-litter size, and delayed ossification in the absence of retarded fetal weight gain. The proportion of early deaths was also increased at 30 mg/kg bw per day (Becker et al., 1991).

In view of the absence of a NOAEL for developmental toxicity in the study of Becker et al. (1991), a supplementary study was conducted in which lower doses of prothioconazole-desthio were used. Groups of 25 naturally-mated young adult female Wistar rats were given prothioconazole-desthio technical (purity, 94.7%) at a dose of 0, 1 or 3 mg/kg bw per day by gavage as a suspension in a 0.5% aqueous Cremophor EL vehicle on days 6 to 15 post coitum. The day of confirmation of mating (when spermatozoa were detected) was designated day 0 of gestation. Mortality, morbidity and clinical signs were recorded twice per day throughout the study. Body weights were recorded daily until sacrifice, starting on day 0 of gestation. Food consumption was recorded for all rats on days 0–6, 6–11, 11–16, and on days 16–21 of gestation. On day 21 of gestation, the rats were killed and assessed for gross pathology. The unopened uteri were weighed, then opened and examined for pregnancy, number and distribution of implantations, live and dead fetuses, and embryonic and fetal

Table 51. Incidences of skeletal variants in a study of developmental toxicity in rats given prothioconazole-desthio by gavage

Skeletal variant	Incidence (%)			
	Dose (mg/kg bw per day)			
	0	10	30	100
No. of fetuses evaluated	156	137	144	125
Incomplete ossification of os occipitale	12 (8)	22 (16)*	23 (16)*	36 (29)**
Unossified cervical vertebra No. 1	23 (15)	26 (19)	36 (25)*	47 (38)**
Incomplete ossification of sternebra:				
No. 1	1 (1)	0 (0)	7 (5)*	12 (10)**
No. 2	42 (27)	33 (24)	59 (41)**	60 (48)**
No. 3	1 (1)	1 (1)	8 (6)	8 (6)**
No. 4	9 (6)	3 (2)	27 (19)**	21 (17)**
No. 5	101 (65)	99 (72)	106 (74)	99 (79)**
No. 6	1 (1)	2 (1)	15 (10)**	26 (21)**
Unossified sternebra No. 6	0 (0)	0 (0)	3 (2)	12 (10)**
One supernumerary rib – left[a]	33 (21)	64 (47)**	108 (75)**	95 (76)**
One supernumerary rib – right[a]	30 (19)	64 (47)**	106 (74)**	93 (74)**
Unossified proximal phalanx – left forelimb:				
Digit 2	65 (42)	41 (30)*	52 (36)	92 (74)**
Digit 5	95 (61)	63 (46)*	85 (59)	106 (85)**
Digit 5 (distal)	56 (36)	45 (33)	44 (31)	26 (21)**
Unossified proximal phalanx – right forelimb:				
Digit 2	61 (39)	38 (28)*	50 (35)	90 (72)**
Digit 5	93 (60)	64 (47)*	83 (58)	107 (86)**
Digit 5 (distal)	53 (34)	47 (34)	43 (30)	20 (16)**
Unossified proximal phalanx – left hindlimb:				
Digit 2	128 (82)	99 (72)	121 (84)	118 (94)**
Digit 3	86 (55)	92 (67)*	120 (83)**	118 (94)**
Digit 4	83 (53)	78 (57)	121 (84)**	116 (93)**
Unossified proximal phalanx – right hindlimb:				
Digit 2	130 (83)	105 (77)	126 (88)	120 (96)**
Digit 3	91 (58)	93 (68)	127 (88)**	123 (98)**
Digit 4	89 (57)	84 (61)	123 (85)**	122 (98)**
Digit 5	143 (92)	125 (91)	137 (95)	124 (99)**

From Becker et al. (1991)

* $p < 0.05$; ** $p < 0.01$.

resorptions (early and late deaths). Non-gravid uteri were stained with 10% ammonium sulfide. The number and distribution of corpora lutea were recorded. Fetuses were weighed, examined for external alterations and the sex determined. Approximately one half of the fetuses from each litter were processed and examined for soft tissue (viscera and brain) alterations by free-hand razor-sectioning. The remaining fetuses were eviscerated, stained with alizarin red S and examined for skeletal alterations. A distinction was made between skeletal abnormalities and variants, the latter being defined as any variation in the number of ribs or the degree of ossification of phalangeal nuclei and/or sternebrae.

There were no effects of treatment on mortality, clinical signs of toxicity, body-weight gain, food consumption or observations of a gross nature at autopsy. There were no adverse effects of treatment on the incidence of pregnancy, numbers of corpora lutea and implantation sites, preimplantation and postimplantation losses, live-litter size, sex ratios and fetal weights. There were statistically significant reductions in the incidences of embryonic deaths and fetal deaths at 3 mg/kg bw per day and fetal weights were statistically significantly higher at both 1 and 3 mg/kg bw per day. Neither of these effects is adverse. No external or visceral malformations occurred in any of the fetuses from dams in the control or treated groups and there were no treatment-related skeletal abnormalities. There were reduced incidences, frequently statistically significant, of fetuses with incomplete ossification of the cranial bones, unossified cervical vertebrae, tali, metatarsals and proximal/distal phalanges in the groups treated at 1 and 3 mg/kg bw per day. These observations were considered to reflect the higher fetal weight, and by implication the more advanced stage of development, at caesarian section of fetuses from treated dams. In contrast, there was a statistically significant increased fetal incidence of supernumerary ribs at 3 mg/kg bw per day (Table 52). In the light of the results of the main study, this change might be considered to be a treatment-related effect. It could also be argued that such an assessment is very conservative, since the incidences at 3 mg/kg bw per day (25–26%) are within the range for historical controls (3–32%) and similar to the incidences in the control group in the main study (19–21%). However, given the similarity of the data in the control group and the group at 1 mg/kg bw per day, the result in the group at 3 mg/kg bw per day is difficult to ignore (Becker & Biedermann, 1991).

The NOAEL for developmental toxicity in rats was 1 mg/kg bw per day on the basis of an increased incidence of supernumerary ribs at 3 mg/kg bw per day. Since there was no distinction between fully-formed supernumerary ribs (a malformation indicating a teratogenic effect) and rudimentary supernumerary ribs (generally considered to be a common skeletal variant) there are doubts as to how best to interpret the data. Consequently, the fetuses from all groups of that study (Becker & Biedermann, 1991) and the control group of the earlier study of Becker et al. (1991) were

Table 52. Incidences of supernumerary ribs in the supplementary study of of developmental toxicity in rats given prothioconazole-desthio by gavage

Parameter	Incidence (%)		
	Dose (mg/kg bw per day)		
	0	1	3
Litters			
No. of litters evaluated	25	24	24
One supernumerary rib – left	11 (44)	12 (50)	15 (63)
One supernumerary rib – right	11 (44)	11 (46)	15 (63)
Fetuses			
No. of fetuses evaluated	146	133	155
One supernumerary rib – left	16 (11)	16 (12)	39 (25)**
One supernumerary rib – right	17 (12)	18 (14)	40 (26)**
Spontaneous fetal incidence in historical controls[a]			
Supernumerary rib – left	Range, 5–32; mean, 15.1; median, 12.5		
Supernumerary rib –right	Range, 3–27; mean, 13.0; median, 13.5		

From Becker & Beidermann (1991a)

[a] Twenty studies conducted between late 1988 and late 1990.

* $p < 0.05$, ** $p < 0.01$

reviewed in a GLP re-evaluation (Klaus, 2004). The results of the re-evaluation are presented in Table 53. Most of the observations were of rudimentary supernumerary ribs. Therefore, although the incidence in the group at 3 mg/kg bw per day remains statistically significant in comparison with the incidence in the concurrent control group, the severity of the effect is lessened. Also, it is unlikely that there would be a significant difference if compared with the main study controls (Klaus, 2004).

In conclusion, the NOAEL for developmental toxicity in rats was 1 mg/kg bw per day on the basis of an increased incidence of rudimentary supernumerary ribs at 3 mg/kg bw per day (Becker & Beidermann, 1991a).

The postnatal fate of supernumerary ribs in rats treated with prothioconazole-desthio was examined. Two groups of 30 naturally-mated young adult female Wistar rats were given prothioconazole-desthio (purity, 93.9%) at a dose of 0 or 30 mg/kg bw per day by gavage as a suspension in 0.5% aqueous Cremophor EL vehicle on days 6 to 15 post coitum. The day of confirmation of mating (when spermatozoa were detected) was designated day 0 of gestation. Half the number of rats in each group was killed on day 20 of gestation and the fetuses were delivered by caesarian section. The remaining dams were allowed to litter normally and rear their young to weaning. Nine additional mated females were introduced into the study, one for caesarian section and eight to rear their young, because of the death of one dam and the high pup mortality in the rearing group. Weaned pups were maintained until day 42–44, at which time they were killed.

Mortality, morbidity and clinical signs were recorded once or twice per day throughout the study. Body weights were recorded on day 0, days 6–15 and on day 20 of gestation. Dams with reared litters were also weighed on postnatal days 0, 7, 14 and 21. Food consumption was recorded for all rats on days 0–6, 6–11, 11–16, and 16–20 of gestation. Gravid uterus weight, numbers of corpora lutea and implantation sites, placental weights and appearance, numbers of live and dead fetuses and fetal weight and sex were recorded at caesarian section. Non-gravid uteri were stained with 10% ammonium sulfide.

All fetuses were examined for external abnormalities, eviscerated, thoracic and abdominal viscera examined fresh for abnormalities, and then processed for skeletal examination as alizarin-stained specimens. The specimens were examined for skeletal abnormalities, delayed ossification and the presence and appearance of supernumerary ribs. The occurrence of 14th rib(s) is classified as a variant because of a high spontaneous occurrence (18%) in the strain employed. Having more than 14 ribs is classified as a malformation owing to a very low spontaneous incidence. Dams allowed to litter normally were killed after weaning of their young. They were additionally assessed for duration of gestation and lactation behavior. The sex and numbers of viable and dead pups were determined shortly after birth. Pup mortality and clinical signs were recorded daily for 6 weeks and body weights were recorded on day 0 and at weekly intervals for 6 weeks. On days 42–44, the progeny were killed and examined for gross pathological changes. The thoraxes were removed and processed for skeletal examination as alizarin-stained specimens. The specimens were examined for the presence and length of supernumerary ribs. Fertility, gestation and rearing indices were calculated for each group. On the basis of the frequency of occurrence in rats in the control groups, 14th rib was classified as a skeletal variant and 15th or 16th ribs were classified as malformations.

There were no treatment-related deaths or clinical signs during the study, on food consumption during gestation, or subsequently during lactation in the dams rearing their young. There were no effects on body-weight gain of rats at 30 mg/kg bw per day during gestation or subsequently during lactation in the dams rearing their young. No treatment-related gross pathological findings were observed in the treated rats either at caesarian section or at the end of the rearing period.

The fertility and gestation indices were unaffected by treatment at 30 mg/kg bw per day. In contrast, the rearing index of this group was markedly depressed, owing to the death of four litters within 3 days of birth and one other litter within 6 days of birth. In the caesarian groups, there was

Table 53. Mean incidences of supernumerary (14th) ribs in studies of developmental toxicity in rats given prothioconazole-desthio in the performing laboratory during 1989 and 1990 [a]

Finding	Dose (mg/kg bw per day)			
	0 (main study)	0 (supplementary study)	1 (supplementary study)	3 (supplementary study)
Number of fetuses (litters) evaluated in original study	156 (25)	146 (25)	133 (24)	155 (24)
Number of fetuses (litters) re-evaluated[b]	38 (17)	17 (11)	19 (12)	43 (15)
Rudimentary ribs				
Percentage of fetuses[c] (litters) with:				
Right 14th rib	1.28 (8.0)	0.68 (4.0)	1.50 (8.3)	1.94 (8.3)
Left 14th rib	5.13 (24.0)	—	0.75 (4.2)	0.65 (4.2)
Bilateral 14th ribs	16.03 (56.0)	10.27 (40.0)	12.03 (41.7)	25.16 (62.5)
Total	22.4 (68.0)	11.0 (40.0)	14.3 (50.0)	27.7* (62.5)
Fully-formed ribs				
Percentage of fetuses[c] (litters) with:				
Right 14th rib	0.64 (4.0)	—	—	—
Left 14th rib	0.64 (4.0)	—	0.75 (4.2)	1.29 (4.2)
Bilateral 14th ribs	—	—	—	—
Total	1.28 (8.0)	—	0.75 (4.2)	1.29 (4.2)
Total rudimentary and fully-formed ribs[c]				
Percentage of fetuses (litters)	22.4 (68.0)	11.0 (40.0)	14.3 (50.0)	27.7* (62.5)
Historical controls[d]				
Percentage fetal incidence:				
Right	Range, 3–27; mean, 14; median, 11			
Left	Range, 3–32; mean, 12; median, 11			
Percentage litter incidence:				
Right	Range, 17–75; mean, 42; median, 42			
Left	Range, 17–83; mean, 46; median, 40			

From Klaus (2004)

[a] Only fetuses with the finding of supernumerary rib in the original studies were re-evaluated for rib length.

[b] Fully-formed 14th ribs only occurred in fetuses with a contralateral rudimentary rib.

[c] Percentage of total fetuses in original study.

[d] Historical-control data for the incidence of 14th ribs from all available studies of embryotoxicity in rats of the same strain treated by oral gavage conducted at RCC (Switzerland) from 1988 to 1990. It does not differentiate between rudimentary and fully-formed ribs or whether some incidences were bilateral.

* $p \leq 0.001$ (compared with concurrent controls from supplementary study).

no effect of treatment on the mean numbers of corpora lutea and implantation sites, the incidences of early and late resorption, live-litter size, fetal weights and sex ratio. However, a statistically significant increase in mean placental weight occurred at 30 mg/kg bw per day. Two placentae in the group were engorged, and six showed a necrotic margin compared with a single incidence in the control group.

The incidence of pups exhibiting retarded ossification of the sternum, ribs and hyoid bone was statistically significantly elevated in the group at 30 mg/kg bw per day (Table 54). Consequently, the total number of fetuses with retarded ossification was also significantly elevated. There was a treatment-related increase in the incidence of fetuses with 14th rib, most of which were punctiform or comma-shaped. All fetuses of the treated group were affected compared with 15.3% of fetuses of the control group.

The malformations, 15th and 16th supernumerary ribs, occurred at frequencies of 3.0% and 1.5% of the fetuses, respectively, in the group at 30 mg/kg bw per day compared with zero incidences in the control group. All 15th and 16th ribs were present as punctiform structures.

Treatment-related malformations of the palate and bones of the forelimbs were identified in fetuses from dams treated at 30 mg/kg bw per day. All fetuses in the group showed dysplasia of the radius and ulna accompanied by dysplasia of the humerus in five fetuses from three litters (Table 55). Ten fetuses from three litters also showed cleft palate resulting in a significantly higher incidence of fetuses with the abnormality.

The duration of gestation for dams in the treated group, 23.0 days, was comparable to the control value of 22.4 days, and there was no evidence of an effect on parturition. Since some pups from four treated litters were not suckled on the day of birth, there may have been an effect of treatment. The number of implantation sites was unaffected by treatment. The mean litter size at birth in the group treated at 30 mg/kg bw per day was slightly lower than the control value, due to three litters of three pups, but the difference from the controls was not statistically significant. However, pup mortality was increased at 30 mg/kg bw per day resulting in a statistically significantly lower survival at weaning. Post-mortem examination of decedent pups and those killed at age 42 days revealed no treatment-related gross lesions, although many decedent pups were not subjected to a full examination owing to advanced autolysis or cannibalism.

Table 54. Incidence of fetal skeletal variants in a study of developmental toxicity in rats given prothioconazole-desthio by gavage

Skeletal variant	Incidence (%)	
	Dose (mg/kg bw per day)	
	0	30
No. of fetuses examined	137	133
Sternum	1 (0.73)	68 (51.1)**
Spine	40 (29.2)	37 (27.8)
Ribs	2 (1.5)	32 (24.1)**
Limbs	1 (0.7)	—
Skull	19 (13.9)	30 (22.6)
Hyoid bone	14 (10.2)	30 (22.6)*
Fetuses with retarded ossification	56 (40.9)	107 (80.5)**
Fetuses with 14th rib	21 (15.3)	133 (100)**

From Holzum (1992b)

* $p < 0.01$; ** $p < 0.001$.

Table 55. Incidence of fetal malformations other than rib defects in a study of developmental toxicity in rats given prothioconazole-desthio by gavage

Malformation	Incidence (%)			
	Dose (mg/kg bw per day)			
	0		30	
	Fetuses	Litters	Fetuses	Litters
No. examined	137	14	133	14
Dysplasia of radius/ulna	0 (0)	0 (0)	133 (100)***	14 (100)***
Dysplasia of humerus	0 (0)	0 (0)	5 (3.8)	3 (21.4)
Cleft palate	0 (0)	0 (0)	10 (7.5)**	3 (21.4)
Total	0 (0)	0 (0)	133 (100)***	14 (100)***

From Holzum (1992b)

** $p < 0.01$; *** $p < 0.001$.

The pup and litter incidences of rudimentary and fully-formed 14th rib at age 42–44 days in the group at 30 mg/kg bw per day were statistically significantly higher than in the concurrent controls. The incidences on the basis of pups were 13.4% and 18.5%, respectively, in the treated group and 3.7% and 0.0%, respectively, in the controls. On a litter basis, the incidences were 18.8% and 56.3%, respectively, in the treated group and 15.4% and 0.0%, respectively, in the controls. However, the incidences of rudimentary 14th rib were markedly (84%) lower in the group of pups at 30 mg/kg bw per day at age 42 days than in fetuses at day 20, while the incidences of fully-formed extra 14th rib were comparable in pups aged 42 days and fetuses at day 20 of gestation. In the control group, the incidence of rudimentary 14th rib in pups aged 42 days also showed a marked (75%) decrease compared with fetuses at day 20 of gestation. Supernumerary 15th and 16th ribs did not occur in any treated or control pups aged 42 days. Thus, the Meeting concluded that the occurrence of fully-formed supernumerary ribs is not reversible after birth, which confirms their classification as a malformation. In contrast, rudimentary (punctiform, comma-shaped) supernumerary ribs are largely reversible after birth, most probably due to merging of the extra ossification point with the vertebral processus lateralis; this confirms their status as simple variations.

The NOAEL for maternal effects was 30 mg/kg bw per day, the highest dose tested. No NOAEL for developmental toxicity could be identified in this study in which a single dose of 30 mg/kg bw per day was used. Adverse effects observed at this dose were: increased pup mortality at birth and neonatal mortality, delayed ossification without a concomitant reduction in fetal weight, and high incidences of long-bone dysplasia and rudimentary 14th supernumerary rib occurred in fetuses at day 20 of gestation, and lower but toxicologically relevant incidences of cleft palate, fully-formed 14th rib and rudimentary supernumerary 15th and 16th ribs. The incidence of rudimentary 14th rib was largely, but incompletely reduced in rats aged 6 weeks (reduction was 75% in controls and 84% in treated pups), while the incidence of fully-formed 14th rib remained unaffected by age. Supernumerary 15th and 16th ribs were absent in rats aged 6 weeks. This reversibility was most probably related to the merging of the extra ossification point with the vertebral processus lateralis and confirms the status of rudimentary supernumerary ribs, which are commonly seen also in untreated rats, as simple variations (Holzum, 1992b).

Groups of 25 naturally-mated young adult female Wistar rats were given prothioconazole-desthio (purity, 93.7%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day by dermal application as a suspension in 1% aqueous Cremophor EL vehicle on days 6 to 15 post coitum. The day of confirmation of mating (when spermatozoa were detected) was designated day 0 of gestation. The applications

were made for 6 h per day, under occlusive dressings, to a 5 × 5 cm area of clipped dorsal skin. The treatment sites were washed with warm water after removal of the dressings. Amongst the other measurements taken, the application sites were examined for dermal irritation according to the Draize scale after each exposure.

No deaths or treatment-related systemic clinical signs occurred at any dose. Transient, minimal reddening of the skin at the application site occurred in one rat in the control group and in one, two and five rats at 100, 300 and 1000 mg/kg bw per day, respectively, and persisted for up to 4 days in one rat at 300 mg/kg bw per day, compared with 1 day in the rat in the control group. Minimal redness in one rat at 1000 mg/kg bw per day was accompanied by transient, minimal swelling.

There were no treatment-related effects on body-weight gain or food and water consumption at any dose. No gross lesions were observed at autopsy at any dose and maternal liver weights were unaffected by treatment.

The incidence of pregnancy and the mean numbers of corpora lutea and implantations were comparable between all test and control groups. Preimplantation and postimplantation losses, live-litter size, placental weight and appearance and fetal sex ratios were unaffected by treatment. Mean fetal weights (for males and females combined) at 100 and 300 mg/kg bw per day were statistically significantly higher than those of the controls, but by < 10% and the absence of a dose–response relationship suggested the differences were unrelated to treatment with prothioconazole-desthio.

The mean number of fetuses per dam and the proportions of fetuses per group showing minor skeletal deviations as a result of retarded ossification were unaffected by treatment at all doses (Table 56). However, there was a statistically significant increase in the occurrence of supernumerary rudimentary 14th ribs in all groups treated with prothioconazole-desthio, although no fully-formed supernumerary 14th ribs were observed. At 1000 mg/kg bw per day, there were minimally-increased incidences of cleft palate, macroglossia, hydrocephaly, hydronephrosis, tubular bone dysplasia and 15th ribs at or above the upper limit of the range for historical controls.

Cases of microphthalmia (two fetuses in two litters each at 300 and 1000 mg/kg bw) could have been an expression of the known high spontaneous inherent biological variability of this finding in this rat strain and not a treatment-related effect for the following reasons. The upper bounds of the range for historical controls for the incidence of microphthalmia in this strain were: fetus basis, 1.95%; litter basis, 20%. The actual maximum incidences in this experiment were 0.94% and 8.7% on fetus and litter bases, respectively. In addition, in the study of developmental toxicity with prothioconazole-desthio administered by gavage to rats of a different strain (Becker et al., 1991), no microphthalmia was reported at doses of up to 100 mg/kg bw per day. Although this oral dose was lower than either of the applied doses at which microphthalmia was observed in the present experiment, it was highly probable that the received internal dose from oral administration would have been similar to, or even higher than, the highest internal dose experienced in this experiment with dermal application. In the kinetic study conducted in pregnant rats (Weber, 2001), it was found that prothioconazole-desthio as multiple oral doses at 1 mg/kg bw per day and as multiple dermal doses at 30 mg/kg bw per day produced similar internal doses (Holzum, 1992a).

The NOAEL for systemic effects in maternal rats was 1000 mg/kg bw per day. No NOAEL was established for intrauterine developmental effects as increased incidences of rudimentary 14th ribs occurred at all doses. The NOAEL for developmental effects excluding rudimentary supernumerary ribs was 300 mg/kg bw per day on the basis of minimally-increased incidences of cleft palate, macroglossia, hydrocephaly, hydronephrosis, tubular bone dysplasia and 15th ribs at 1000 mg/kg bw per day (Holzum, 1999).

In a study designed to identify a NOAEL for supernumerary ribs in rats, groups of 25 naturally-mated young adult female Wistar rats were given prothioconazole-desthio (purity, 94.0–94.7%) at a

Table 56. Summary of fetal and litter incidence of abnormalities in a study of developmental toxicity in rats given prothioconazole-desthio dermally

Abnormality	Fetal incidence (litter incidence)			
	Dose (mg/kg bw per day)			
	0	100	300	1000
No. of litters evaluated	23	21	23	24
Total No.of fetuses evaluated	222	200	229	213
No. of fetuses evaluated (visceral)	105	96	109	101
No. of fetuses evaluated (skeletal)	117	104	120	112
Cleft palate	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.47 (4.17)
Macroglossia	0.45 (4.35)	0.0 (0.0)	0.0 (0.0)	3.29 (4.17)
Hydrocephaly	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.47 (4.17)
Microphthalmia	0 (0.0)	0 (0.0)	0.87 (8.70)	0.94 (8.33)
Hydronephrosis, hydroureter	0.45 (4.35)	0.50 (4.76)	0 (0.0)	1.41 (8.33)
Supernumerary rudimentary 14th rib	3.2 (17)	22 (81)***	27 (83)***	29 (88)***
Supernumerary 15th rib	0 (0.0)	0 (0.0)	0 (0.0)	0.47 (4.17)
Tubular bone dysplasia	1.35 (4.35)	0 (0.0)	1.31 (13.04)	2.82 (8.33)
No. of abnormal fetuses per group	9	2	7	13
No. of abnormal fetuses (mean per dam)	0.39	0.10	0.30	0.54
Abnormal fetuses (% per group)	4.1	1.0	3.1	6.1
No. of litters with abnormal fetuses	5	2	4	5
Abnormal litters (% per group)	21.7	9.5	17.4	20.8

From Holzum (1999)

*** $p < 0.001$

dose of 0 10 or 30 mg/kg bw per day by dermal application as a suspension in 1% aqueous Cremophor EL vehicle on days 6 to 15 post coitum. The day of confirmation of mating (when spermatozoa were detected) was designated day 0 of gestation. The applications were made for 6 h per day, under occlusive dressings, to a 5 × 5 cm area of clipped dorsal skin. The treatment sites were washed with warm water after removal of the dressings. Amongst the other measurements taken, the application sites were examined for dermal irritation according to the Draize scale after each exposure.

No deaths or treatment-related systemic clinical signs occurred at either dose. Transient, minimal reddening of the skin at the application site occurred in three rats in the control group and in six and four rats treated at 10 and 30 mg/kg bw per day, respectively, that persisted for up to 8 days in one rat at 30 mg/kg bw per day, compared with 1 day in the rat in the control group. Therefore, treatment-related local irritation coud not be excluded at 30 mg/kg bw per day.

There were no treatment-related effects on body-weight gain or food and water consumption. No treatment-related gross lesions were observed and maternal liver weights were unaffected by treatment.

The incidences of pregnancy and the mean numbers of corpora lutea and implantations were comparable between all test and control groups. Preimplantation and postimplantation losses, live-litter size, mean fetal weight, mean placental weight and appearance and fetal sex ratios were unaffected by treatment.

The mean number of fetuses per dam and the proportion of fetuses per group showing minor skeletal deviations as a result of retarded ossification were unaffected by treatment. Similarly, the

percentages of fetuses with 14th rib, either punctiform or comma-shaped, in the group at 10 mg/kg bw per day (13%) and the group at 30 mg/kg bw per day (18%) were comparable to the incidence of 18% in the control group. No fully-formed supernumerary 14th ribs were seen. Treatment at doses of 10 and 30 mg/kg bw per day did not influence the nature and incidence of fetal abnormalities (Table 57).

The NOAEL for systemic effects in maternal rats was 30 mg/kg bw per day on the basis of the absence of maternal systemic effects at the highest dose tested. The NOAEL for developmental effects, and specifically, the incidence of rudimentary 14th rib, was 30 mg/kg bw per day, the highest dose tested, on the basis of the absence of intrauterine developmental effects at this dose (Bartmann, 1991).

Rabbits

Groups of 15 mated female Chinchilla rabbits were given prothioconazole-desthio (purity, 94.0–94.7%) at a dose of 0, 2, 10 or 50 mg/kg bw per day by gavage in 0.5% aqueous Cremophor EL from day 6 to day 18 post coitum. The doses for this study were based on the results of a preliminary study in which doses of 30, 50 and 75 mg/kg bw per day were testeded. The rabbits showed marked body-weight loss and increased incidence of resorptions at 50 mg/kg bw per day and total resorption at 75 mg/kg bw per day. In addition to the parameters expected by current guidelines, maternal livers were weighed and samples retained for microscopic examination.

There were no deaths and no treatment-related clinical signs. The mean food consumption of the group treated at 50 mg/kg bw per day was non-significantly lower by 11.4% relative to that of the controls during the treatment period and subsequently. Presumably due to the elevated resorption rate, the group of rabbits at 50 mg/kg bw per day lost body weight during treatment and gestation. Body-weight gain was unaffected by treatment at 2 and 10 mg/kg bw per day.

At post mortem, no treatment-related gross lesions were identified and there was no effect on maternal liver weight at any dose. However, treatment-related histopathological effects in the liver

Table 57. Incidence of fetal abnormalities in a study of developmental toxicity in rats given prothioconazole-desthio dermally

Abnormality	Fetal (litter) incidence		
	Dose (mg/kg bw per day):		
	0	10	30
No. of litters evaluated	19	20	19
Total No. of fetuses evaluated	170	196	177
No. of fetuses evaluated (visceral)	81	92	83
No. of fetuses evaluated (skeletal)	89	104	94
Humerus dysplasia	0.59 (5.26)	0 (0.0)	0 (0.0)
Microphthalmia	0.59 (5.26)	0 (0.0)	0 (0.0)
Vertebral arches missing, kinked spine/tail, asymmetric vertebral bodies/arches	0 (0.0)	0.51 (5.0)	0 (0.0)
Total No. of abnormal fetuses per group	2	1	0
Mean No. of abnormal fetuses per dam	0.11	0.05	0.0
Abnormal fetuses per group (%)	1.18	0.51	0.0
No. of litters with abnormal fetuses	2	1	0
Abnormal litters per group (%)	10.5	5.0	0.0

From Bartmann (1991)

were observed at doses of 10 and 50 mg/kg bw per day (Table 58). The effects were characterized by an increased incidence and intensity of focal round-cell infiltrations, mainly nodular Kupffer cell proliferation, and the occurrence of optically denser cytoplasm of hepatocytes suggestive of reduced intracellular glycogen concentrations. Two rabbits at 50 mg/kg bw per day also showed mild centrilobular hypertrophy.

There was no effect on the numbers of corpora lutea and implantations at any dose, but the postimplantation loss in all treated groups was statistically significantly higher than in the controls (Table 59). However, since the incidences at 2 and 10 mg/kg bw per day were within the range for historical background variation (0.3–2.2 resorptions per doe in 13 studies carried out in 1985–1990), the differences from the control group may not have been related to treatment at these doses. As a consequence of the treatment-related increase in resorption incidence at 50 mg/kg bw per day, the mean number of live fetuses was significantly reduced. The sex ratios, fetal weights and placental weights were unaffected by treatment at any dose.

There were no treatment-related effects on the incidences of minor skeletal deviations comprising centres of absent, retarded or incomplete ossification of individual bones or skeletal sections.

Table 58. Incidence of treatment-related histopathological alterations of the liver in a study of developmental toxicity in rabbits given prothioconazole-desthio by gavage

Liver alteration	Incidence (%) and [mean severity]			
	Dose (mg/kg bw per day)			
	0	2	10	50
Enhanced stainability	20 [1.3]	27 [1.3]	50 [1.6]	75 [1.9]
Focal round-cell infiltration	27 [1.3]	33 [1.0]	64 [1.4]	67 [1.5]
Hypertrophy	0 [—]	0 [—]	0 [—]	17 [1.5]

From Bartmann (1992)

Table 59. Group mean litter data in a study of developmental toxicity in rabbits given prothioconazole-desthio by gavage

Parameter	Dose (mg/kg bw per day):			
	0	2	10	50
No. pregnant/No. tested	15/15	15/15	14/15	12/15
Corpora lutea (mean per doe)	8.3	8.4	7.5	8.3
Implantations (mean per doe)	5.6	6.9	5.9	6.8
No. of live fetuses (mean per doe)	5.1	5.2	4.7	3.1*
No. of dead fetuses	0	0	0	0
Early resorptions (mean per doe)	0.13	0.6	0.64	1.83
Late resorptions (mean per doe)	0.33	1.07	0.57	1.83
Postimplantation loss (mean per doe)	0.5	1.7**	1.2*	3.7***
Sex ratio (males : females)	1 : 1.03	1 : 1.17	1 : 1.36	1 : 1.06
Live fetal weight (g) [males + females] (mean per group)	42.2	42.1	41.0	41.3
Placental weight (g) (mean per group)	5.20	4.78	4.82	5.14

From Bartmann (1992)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

There were, however, treatment-related increases in the incidence of abnormal fetuses at doses of 10 and 50 mg/kg bw per day (Table 60). The abnormalities observed at 10 mg/kg bw per day occurred in 10.6% of fetuses in 38.4% of litters and consisted of arthrogryposis, multiple abnormalities or vertebral body/rib alterations. Arthrogryposis and multiple abnormalities have occurred spontaneously in rabbits of this strain in control groups (and arthrogryposis was not clearly dose-related and is a common malformation in rabbits most likely due to restricted fetal movement in the uterus that is considered to be largely reversible after birth). Nevertheless, the incidences at 10 mg/kg bw per day were higher than background, so an effect of treatment could not be discounted. At 50 mg/kg bw per day, there was an unequivocal treatment-related incidence of 13.5% fetuses with cleft palate, indicating that prothioconazole-desthio has a teratogenic effect. The incidence of abnormal fetuses and litters at 2 mg/kg bw per day was unaffected by treatment.

The NOAEL for maternal rabbits was 2 mg/kg bw per day on the basis of the occurrence of histomorphological alterations in the liver at 10 mg/kg bw per day and decreased food consumption and body-weight loss at 50 mg/kg bw per day. The NOAEL for developmental effects was also 2 mg/kg bw per day on the basis of the occurrence of increased incidences of abnormal fetuses at 10 mg/kg bw per day and increased postimplantation loss at 50 mg/kg bw per day (Bartmann, 1992).

4.6 Special studies

(a) Delayed neurotoxicity

No data were submitted that would address possible delayed neurotoxicity. Since prothioconazole-desthio is not a member of a chemical class associated with delayed neurotoxicity and since there was no evidence of changes in nervous tissues, testing for delayed neurotoxicity was not required.

Table 60. Incidence of fetal abnormalities in a study of developmental toxicity in rabbits given prothioconazole-desthio by gavage

Abnormality	Incidence in fetuses			
	Dose (mg/kg bw per day)			
	0	2	10	50
No. of fetuses examined	77	78	66	37
Cleft palate	0	0	0	5
Forked rib	0	1	0	0
Floating rib	1	0	0	0
Vertebral body and rib alterations	0	0	1	0
Arthrogryposis	0	1	5	1
Shortened tail	0	0	0	1
Multiple abnormalities	0	0	2[a]	0
Occlusion of nostrils	1	0	0	0
Total No. (%) abnormal fetuses	2 (2.6)	2 (2.5)	7 (10.6)	7* (18.9)
Abnormal fetuses (mean per doe)	0.13	0.14	0.54	0.70**
Total No. (%) litters with abnormal fetuses	2 (13.3)	2 (14.2)	5 (38.4)	3 (30.0)

From Bartmann (1992)

* $p < 0.05$; ** $p < 0.01$

[a] One fetus with arthrogryposis, one shortened limb, double-length tail, umbilical hernia and cleft spine, one fetus with generalized oedema, shortened lower jaw, bilateral cleft lip, dysplasia of eyes and severe lung dysplasia.

(b) Neurotoxicity

Groups of mated female Wistar rats (strain: Wistar Hannover Crl:WI (GlxBRL/Han) IGS BR) designed to provide a minimum of 20 litters per group were given diets containing prothioconazole-desthio (purity, 99.1–99.4%) at a concentration of 0, 40, 160 or 500 ppm from day 6 of gestation to day 21 of lactation. These dietary concentrations were equal to doses during gestation of 0, 3.6, 15.1 and 43.3 mg/kg bw per day, and to doses during lactation of 0, 8.1, 35.7 and 104.6 mg/kg bw per day.

On postnatal day 4, litters with a minimum of eight pups, including at least three males and three females, were culled to yield, as closely as possible, four males and four females. Subsets of surviving offspring, representing at least 20 litters per group, were subjected to evaluation using the following observations and measurements: detailed clinical observations and a functional observational battery (FOB), preputial separation or vaginal patency, body weight, automated measures of activity (figure-eight maze), acoustic startle habituation, learning and memory (passive avoidance after weaning and a water-maze task beginning on postnatal day 60 ± 2 days) and an eye examination. Neural tissues were collected from 10 males and 10 females per group (representing approximately 20 litters) on postnatal day 21 (brain only) and at study termination (age approximately 75 days) for microscopic examination and morphometry.

Mortality was not affected by treatment and no treatment-related clinical signs were observed. Three dams at the highest dose were killed on day 22 of gestation due to dystocia. Body weights, body-weight gain and food consumption were not affected. FOB investigations revealed no treatment-related findings.

Exposure to diets at 500 ppm caused a decreased fertility index, an increased duration of gestation, fetal deaths, and three dams in dystocia with dead fetuses on day 22 of gestation. In addition to the dead pups in three females at the highest dose during late pregnancy, the number of stillborn pups was slightly increased in the groups at higher doses. The numbers of stillborn pups were 0, 0, 2 and 3 in the groups at 0, 40, 160 and 500 ppm, respectively. The occurrence of two stillborn pups in the group at 160 ppm was not necessarily an effect of treatment as it was within the range for historical controls.

Pup birth weight, body-weight gain and terminal body weight were not affected. There were no treatment-related effects on preputial separation or vaginal opening at any dietary concentration.

Detailed clinical observations revealed the development of a deviated snout with associated malocclusion of the incisors in the groups of pups at 160 and 500 ppm. This observation was not made until after weaning, as the snout elongated with maturation and lateral deviation from the mid-line became progressively more severe. Deviated snout and associated malocclusion are considered to be less severe manifestations related to improper development of the palate, while cleft palate seen in previously conducted studies of developmental and reproductive toxicity with prothioconazole-desthio is regarded as a more severe effect. Deviated snout and cleft palate are therefore assessed as interrelated expressions of an insult on skull mid-line sutures. Associated findings, like lacrimation and lacrimal stain, were also observed in some of the rats exhibiting deviated snout and malocclusion in the present study. Gross postmortem examination identified additional rats in the groups at 160 and 500 ppm with associated skull findings (e.g. ulceration of the dorsal palate, missing dorsal incisors, nasal-bone fracture).

FOB investigations confirmed the malocclusion and associated findings in groups of rats at 160 and 500 ppm that had also been observed during clinical observations. All other parameters investigated (motor and locomotor activity, acoustic startle habituation, passive avoidance, water-maze task, pupil constriction, ophthalmology) were not affected at any dietary concentration. On postnatal day 21 or at study termination, no treatment-related effects were observed on brain weight, gross or microscopic brain measurements, or on neuropathological parameters (microscopic investigations on brain, neural tissues and skeletal muscle).

The NOAEL for maternal and reproductive toxicity was 160 ppm, equal to 15.1 mg/kg bw per day during gestation, on the basis of dystocia and increased duration of gestation observed at 500 ppm, equal to 43.3 mg/kg bw per day during gestation. The NOAEL for neonatal toxicity was 40 ppm, equal to 3.6 mg/kg bw per day during gestation, on the basis of the occurrence of deviated snout, malocclusion of the incisors and associated skull findings (ulceration of the dorsal palate, missing dorsal incisors and/or nasal bone fracture) at 160 ppm, equal to 15.1 mg/kg bw per day during gestation. These effects were assessed as expressions of an insult on mid-line skull sutures that are less severe than (but interrelated with) the formation of cleft palate which had been observed in previously conducted studies of developmental and reproductive toxicity conducted with prothioconazole. The NOAEL for developmental neurotoxicity was 500 ppm, equal to 43.3 mg/kg bw per day during gestation, on the basis of the absence of effects on neurobehavioral and learning and memory parameters, on brain weight, brain morphometry and on neuropathology parameters at this dose, the highest tested (Sheets & Lake, 2004).

5. Studies with other metabolites of prothioconazole

The following are metabolites that have been found in wheat and on which toxicological studies have been conducted:

— Prothioconazole-sulfonic acid (M02, JAU 6476-SA) is a major metabolite found in the rotational wheat straw or hay. It was not identified in studies of metabolism in rats.

— Prothioconazole-*alpha*-hydroxy-desthio (M18), found in wheat, but not in studies of metabolism in rats.

— Prothioconazole-*alpha*-acetoxy-desthio (M19), found in wheat, but not in studies of metabolism in rats.

— Prothioconazole-benzylpropyldiol (M09) found in wheat, but not in studies of metabolism in rats.

— Prothioconazole-triazolinone (M03) occurred in trace quantities in rats, but it was a substantial metabolite in fodder wheat.

5.1 Prothioconazole-sulfonic acid (M02)

(a) Acute toxicity

The acute oral toxicity of prothioconazole-sulfonic acid was examined in groups of three male and three female Wistar rats. All rats died between 5 h and 2 days after receiving a dose of 2000 mg/kg bw, while all rats receiving a dose of 200 mg/kg bw survived for 14 days (Kroetlinger, 2000a). There have been no other studies of oral toxicity, of toxicity by other routes, or of dermal or ocular irritation or sensitization potential.

(b) Short-term studies of toxicity

In a 90-day feeding study, groups of Wistar rats were given diets containing prothioconazole-sulfonic acid (potassium salt) (purity, 98.9%; + 1% peanut oil) at constant nominal concentrations of 0, 30, 125, 500 or 2000 ppm for 13 weeks. Mean achieved doses were 0, 2.1, 8.7, 34.3 and 136 mg/kg bw per day for males and 0, 2.6, 9.7, 40.4 and 163 mg/kg bw per day for females. There were no treatment-related premature deaths and no treatment-related clinical signs at any dose. Body-weight gain was unaffected by treatment at any dose. The food consumption of females in groups treated with prothioconazole-sulfonic acid at ≥ 125 ppm was slightly reduced during week 1 only and did not influence body-weight gain.

There were no treatment-related effects on haematology, blood chemistry (which included measurement of the thyroid hormones T4, T3 and TSH) or urine-analysis parameters. There were no treatment-related effects on hepatic P450-dependent monooxygenase enzyme activities and triglyceride concentrations in either sex at any dose. In the group of males treated at 2000 ppm, EH, GST and GLU-T activities were increased by 66%, 28% and 22%, respectively. GST activity was also minimally increased by 20–28% in males at ≥ 500 ppm. The minor effect on hepatic-enzyme activities was not considered to be adverse since liver weight and histomorphology were not affected by treatment.

There were no treatment-related gross pathological findings at necropsy or effects on organ weights in any treatment group. The only dose-related, statistically significant difference in absolute and relative organ weights was the mean weight of the heart of females at 2000 ppm, which was 10% lower than the control value. In the absence of a histopathological correlate, the observation was considered to be incidental to treatment; however, a similar response was observed with prothioconazole itself. Treatment-related histopathological changes were confined to minimal to moderate transitional-cell hyperplasia in the urinary bladder of four males at 2000 ppm. The effect was not apparent in any of the females at 2000 ppm or in any males or females at lower doses. The nature and incidence of all other histopathological findings did not suggest an effect of treatment at any dose.

The NOAEL was 500 ppm, equal to 34 mg/kg bw per day, on the basis of the occurrence of histomorphological alterations in the urinary bladder of males at 2000 ppm, equal to 136 mg/kg bw per day (Andrews & Hartmann, 2001).

(c) Genotoxicity

Prothioconazole-sulfonic acid was tested for mutagenic activity in *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 in standard plate tests and preincubation test assays, both in the presence and absence of an exogenous metabolic activation system prepared from a post-mitochondrial supernatant from male rat liver. The experiments were duplicated. There was no evidence for the induction of gene mutations in bacteria by prothioconazole-sulfonic acid at concentrations up to 5000 µg/plate or tube, at which a weak bacteriotoxic effects occurred. Positive controls produced the predicted results (Herbold, 2000d).

(d) Developmental toxicity

In a non-GLP, non-guideline dose range-finding study of developmental toxicity, groups of seven female Wistar rats were given prothioconazole-sulfonic acid (potassium salt) (purity, 99.3%) at a dose of 0, 30, 100, 500 or 1000 mg/kg bw per day by gavage in bi-distilled water from day 6 to day 20 of gestation. The day of observed mating was designated day 0 of gestation. Rats were observed twice per day for morbidity, mortality and clinical signs of a reaction to treatment. Individual food consumption was recorded for 3- or 4-day intervals and body weights were recorded daily throughout gestation until caesarean section. Decedents were subjected to post-mortem investigations. Survivors were killed on day 21 post coitum and subjected to gross examination and autopsy in which the gravid uterus was weighed and the fetuses were removed. The position of fetuses in the uterine horns, the numbers of implantation sites and corpora lutea, fetal weights and sexes were recorded. The fetuses were examined for external malformations. Implantation sites were classified as embryonic resorption, fetal resorption, dead fetus or live fetus. If no implantation sites were evident, the uterus was immersed in ammonium sulfide solution to aid visualization. Samples of abnormal maternal tissues were retained in fixative. All fetuses were killed, processed by the Dawson technique to visualize the ossified skeleton and then examined for skeletal abnormalities and variations. Soft tissues were not examined for abnormalities or malformations.

Five of the seven females treated at 1000 mg/kg bw per day died on days 9–11 post coitum having received three to five doses of prothioconazole-sulfonic acid. The two remaining females in the group were killed for humane reasons on day 10 or day 12 post coitum. Before death or euthanasia,

the rats showed body-weight loss, an approximate 50% reduction in food consumption and ruffled fur and sedation. There were no maternal deaths, treatment-related clinical signs or changes in body-weight gain and food consumption at any lower doses up to 500 mg/kg bw per day. Reproductive parameters of the group treated at 1000 mg/kg bw per day were not evaluated because of the early stage of gestation at which death occurred. There were no treatment-related effects on reproductive parameters at the other doses. Post-mortem examination of the dams did not show any treatment-related gross lesions at any dose.

No external fetal abnormalities occurred in any group. The incidence and nature of skeletal abnormalities did not indicate an effect of treatment at any dose. Similarly, the distribution of skeletal variations did not indicate an effect of treatment at any dose. Although there were statistically significant ($p < 0.05$ or 0.01) differences between the control group and the group at 500 mg/kg bw per day, there was no consistent evidence of retarded ossification since the significant incidences at 500 mg/kg bw per day varied from the controls in both directions.

The NOAEL for maternal toxicity was 500 mg/kg bw per day on the basis of maternal deaths at 1000 mg/kg bw per day. The NOAEL for developmental effects in this dose range-finding study was 500 mg/kg bw per day, the highest dose at which assessment could be made (Becker & Marburger, 2001).

In a study of developmental toxicity, four groups of 25 mated female Wistar rats were given prothioconazole-sulfonic acid (potassium salt) (purity, 98.9%) at a dose of 0, 30, 150, or 750 mg/kg bw per day by gavage in bi-distilled water daily from day 6 to day 20 of gestation. The day that sperm occurred in a vaginal smear, or the presence of a copulation plug, was designated day 0 of gestation. The rats were observed twice per day for morbidity, mortality and clinical signs of a reaction to treatment. Individual food consumption was recorded at 5- or 6-day intervals and body weights were recorded daily throughout gestation until caesarean section. Premature decedents were subjected to a post-mortem examination. The surviving rats were killed and subjected to autopsy on day 21 of gestation, the gravid uterus was weighed and the fetuses were removed. The position of fetuses in the uterine horns, the numbers of implantation sites and corpora lutea, fetal weights and sexes were recorded. The fetuses were examined for external malformations. Implantation sites were classified as embryonic resorption, fetal resorption, dead fetus or live fetus. If no implantation sites were evident, the uterus was immersed in ammonium sulfide solution to aid visualisation. Samples of abnormal maternal tissues were retained in fixative. All fetuses were killed, and approximately one half of the fetuses from each litter were fixed in Bouin fluid for examination by a microdissection technique comprising serial sectioning of the head and microdissection of the thorax and abdomen. The remaining fetuses were processed by a double-staining technique to visualize the ossified and cartilaginous skeleton and then examined for skeletal abnormalities and variations.

There were no deaths at doses up to 150 mg/kg bw per day, but seven treatment-related deaths occurred at 750 mg/kg bw per day, six between days 9 and 12 post coitum and one on day 21. Treatment-related clinical signs were confined to rats treated at 750 mg/kg bw per day and comprised ruffled fur. Two rats also developed irregular or noisy breathing. Treatment-related effects on food consumption were confined to the group treated at 750 mg/kg bw per day in which there was a statistically significant 16.4% decrease, relative to the control group, in overall food consumption during the treatment period. All dams in the group showed reduced food consumption on days 6–11, all except five on days 11–16 and all except seven dams on days 16–21. The group mean body-weight gain of dams at 750 mg/kg bw per day was markedly reduced during the entire treatment period and a mean body-weight loss of 6 g occurred during the first 2 days (6–8) of treatment. Thus, on day 21 the group mean body weight at 750 mg/kg bw per day was 5.3% lower than that of the control group, although it had been 5.3% higher at day 6. There were no treatment-related gross findings at autopsy of the dams from any treatment groups. Three dams at 750 mg/kg bw per day showed complete

postimplantation loss and there was total fetal resorption in another dam. Total postimplantation loss occurred in one dam in each of the other groups, including the control group. No effects were observed at autopsy in dams with live fetuses on the postimplantation loss and litter number at any dose. There were no treatment-related effects at any dose on litter parameters except for mean pup weights at 750 mg/kg bw per day, which were significantly reduced by 12.2 and 14.9% for male and female pups, respectively. Fetal weights at lower doses were not affected by treatment.

There were no external or visceral abnormalities at any dose that were considered to be treatment-related (Table 61). Skeletal abnormalities occurred in one, two, three and seven fetuses from one, two, three and five litters, respectively, in the groups at 0, 30, 150 and 750 mg/kg bw per day, respectively. The nature and incidences of the abnormalities (asymmetrically ossified sternebrae, wavy ribs and abnormally ossified vertebral bodies) were considered to be incidental to treatment because the numbers of fetuses affected by each individual anomaly were within the ranges for historical controls. A retarded skeletal ossification, correlating with the reduced fetal weights, was observed at 750 mg/kg bw per day. The incidence of supernumerary rudimentary 14th rib was statistically significantly increased in the groups at 30 and 150 mg/kg bw per day. However, the incidence at 750 mg/kg bw per day was lower than in these two groups and was only marginally higher than, and not statistically significantly different from, the incidence in the control group. Since the differences from the controls were not dose-related and all group incidences fell within the ranges for the historical controls 1.4–21.2% fetuses (left) and 0.7–19.2% fetuses (right) for supernumerary 14th rib, the differences were not considered to be related to treatment.

The NOAEL for maternal toxicity with prothioconazole-sulfonic acid was 150 mg/kg bw per day on the basis of the occurrence of increased mortality, reduced food consumption and body-weight gain at 750 mg/kg bw per day. The NOAEL for fetotoxicity for prothioconazole-sulfonic acid was 150 mg/kg bw per day on the basis of an increased incidence of total postimplantation loss, the occurrence of

Table 61. Summary of abnormalities and selected skeletal variants in a study of developmental toxicity in rats given prothioconazole-sulfonic acid by gavage

Abnormality or skeletal variant	Dose (mg/kg bw per day)			
	0	30	150	750
No. of litters examined	23	24	20	15
No. of fetuses examined (external)	264	248	230	193
No. (%) of externally abnormal fetuses	0 (0.0)	0 (0.0)	1 (0.43)	1 (0.52)
No. of fetuses examined (visceral)	123	120	108	93
No. (%) of fetuses abnormal (visceral)	32 (26.02)[a]	33 (27.50)[b]	27 (19.44)[c]	14 (15.05)[d]
No. of fetuses examined (skeletal)	141	128	122	100
No. (%) of fetuses abnormal (skeletal)	1 (0.71)	2 (1.56)	3 (2.46)	7 (7.00)
No. (%) of fetuses abnormal (cartilage)	1 (0.71)	4 (3.13)	2 (1.64)	1 (1.00)
No. (%) with 14th left rib	2 (1.42)	0 (0.0)	0 (0.0)	2 (2.00)
No. (%) with 14th left rib (rudimentary)	6 (4.25)	19 (14.84)**	19 (15.57)**	8 (8.00)
No. (%) with 14th right rib	2 (1.42)	0 (0.0)	0 (0.0)	0 (0.0)
No. (%) with 14th right rib (rudimentary)	8 (5.67)	15 (11.72)	18 (14.75)*	11 (11.00)

From Becker et al. (2001)

* $p \leq 0.05$; ** $p \leq 0.01$.

[a] Twenty-one fetuses with left-sided umbilical artery only.

[b] Twenty-six fetuses with left-sided umbilical artery only.

[c] Sixteen fetuses with left-sided umbilical artery only.

[d] Eight fetuses with left-sided umbilical artery only.

retarded fetal weight gain and ossification at 750 mg/kg bw per day. Prothioconazole-sulfonic acid did not show any teratogenic potential at doses up to 750 mg/kg bw per day (Becker et al., 2001).

5.2 Prothioconazole-triazolinone (M03)

(a) Acute toxicity

The acute oral toxicity of prothioconazole-triazolinone was examined in groups of three male and three female Wistar rats given a dose of 2000 mg/kg bw by gavage. One rat died, but all others survived for 14 days. Clinical signs of toxicity were decreased mobility and reactivity, uncoordinated gait and laboured breathing in males and females, and lateral and/or abdominal position in some males. In addition, there were isolated occurrences of piloerection, spastic/creeping gait, increased salivation and narrow palpebral fissures in females. Clinical signs were first apparent within 45 min after treatment and some persisted up to day 5. Body-weight gain was unaffected by treatment and there were no treatment-related gross lesions at autopsy either in the decedent or in the survivors killed at the end of the observation period. The acute oral LD_{50} of prothioconazole-triazolinone in rats is > 2000 mg/kg bw (Kroetlinger, 2000b).

There have been no other studies of oral toxicity or any studies of toxicity by other routes, or of dermal or ocular irritation or sensitization potential with prothioconazole-triazolinone.

(b) Genotoxicity

JAU 6476-triazolinone was tested for mutagenic activity in *S typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 in standard plate-test and preincubation-test assays, both in the presence and absence of an exogenous metabolic activation system prepared from a post-mitochondrial supernatant from male rat liver. The experiments were duplicated. There was no evidence for the induction of gene mutations in the bacteria by prothioconazole-triazolinone at concentrations up to 500 µg/plate or tube, at which a bacteriotoxic effect occurred. Positive controls produced the predicted results (Herbold, 2000b).

5.3 Prothioconazole-alpha-hydroxy-desthio (M18)

(a) Acute toxicity

The acute oral toxicity of prothioconazole-alpha-hydroxy-desthio was examined in Wistar rats (three per sex) treated by gavage at 2000 mg/kg bw. There were no deaths during the study. Clinical signs of a reaction to treatment, decreased mobility and reactivity, uncoordinated gait, laboured breathing, piloerection and narrowing of the palpebral fissures, occurred in males and females. Clinical signs were first apparent within 60 min of treatment and some persisted up to day 8. One female showed slight weight loss during the first week of observation, but subsequently gained weight. The body-weight gain of all other animals was unaffected by treatment and there were no gross lesions evident at autopsy. The acute oral LD_{50} of prothioconazole-alpha-hydroxy-desthio in the rat is > 2000 mg/kg bw (Kroetlinger, 2000c).

There have been no other studies of oral toxicity or any studies of toxicity by other routes, or of dermal or ocular irritation or sensitization potential with prothioconazole-alpha-hydroxy-desthio.

(b) Genotoxicity

Prothioconazole-alpha-hydroxy-desthio was tested for mutagenic activity in *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 in standard plate-test and preincubation-test assays, in the presence and absence of an exogenous metabolic activation system prepared from a

post-mitochondrial supernatant from male rat liver. The experiments were duplicated. There was no evidence for the induction of gene mutations in bacteria by prothioconazole-alpha-hydroxy-desthio at concentrations of up to 5000 μg/plate or tube, at which a weak bacteriotoxic effect occurred. Positive controls produced the predicted results (Herbold, 2000a).

5.4 Prothioconazole-alpha-acetoxy-desthio

(a) Acute toxicity

The acute oral toxicity of prothioconazole-alpha-acetoxy-desthio was examined in groups of three male and three female Wistar rats given a dose of 2000 mg/kg bw by gavage. There were no deaths during the study. Clinical signs of a reaction to treatment, decreased mobility and reactivity, uncoordinated gait, laboured breathing and piloerection occurred in males and females. One male rat also showed narrowing of the palpebral fissure. Clinical signs were first apparent within 45 min of treatment and some persisted up to day 8. One female showed slight weight loss during the first week of observation, but subsequently gained weight. The body-weight gain of all other rats was unaffected by treatment and there were no gross lesions evident at necropsy. The acute oral LD_{50} of prothioconazole-alpha-acetoxy-desthio in rats was > 2000mg/kg bw (Kroetlinger, 2000d).

There have been no other studies of oral toxicity or any studies of toxicity by other routes, or of dermal or ocular irritation or sensitization potential with prothioconazole-alpha-acetoxy-desthio.

(b) Genotoxicity

Prothioconazole-alpha-acetoxy-desthio was tested for mutagenic activity in *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 in standard plate-test and preincubation-test assays, in the presence and absence of an exogenous metabolic activation system prepared from a post-mitochondrial supernatant from male rat liver. The experiments were duplicated. There was no evidence for the induction of gene mutations in bacteria by prothioconazole-alpha-acetoxy-desthio at concentrations of up to 5000 μg/plate or tube, at which bacteriotoxic effects occurred. Positive controls produced the predicted results (Herbold, 2000c).

5.5 Prothioconazole-benzylpropyldiol (M09)

(a) Acute toxicity

The acute oral toxicity of prothioconazole-benzylpropyldiol was examined in groups of three male and three female Wistar rats given a dose of 2000 mg/kg bw by gavage. There were no deaths during the study. Clinical signs of a reaction to treatment were confined to female rats that showed a moderate to marked increase in salivation between 5 and 30 min after treatment. Thereafter, no other clinical signs of toxicity were recorded. The body-weight gains of males and females were unaffected by treatment and no gross lesions were observed at autopsy in any rat. The acute oral LD_{50} of prothioconazole-benzylpropyldiol in rats was > 2000mg/kg (Kroetlinger, 2000e)

(b) Genotoxicity

Prothioconazole-benzylpropyldiol was tested for mutagenic activity in *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 in standard plate-test and preincubation-test assays, in the presence and absence of an exogenous metabolic activation system prepared from a post-mitochondrial supernatant from male rat liver. The experiments were duplicated. There was no evidence for the induction of gene mutations in bacteria by prothioconazole-benzylpropyldiol at concentrations of up to 256 μg/plate or tube, at which bacteriotoxic effects occurred. Positive controls produced the predicted results (Herbold, 2000e).

6. Observations in humans

6.1 Medical surveillance of manufacturing-site personnel

Prothioconazole being a new compound, there was little information available on exposure of workers and none on the general population. No symptoms have been reported from the handling of prothioconazole during synthesis, formulation or during process development. No other information was available.

Comments

Prothioconazole

Biochemical aspects

In rats given [^{14}C]prothioconazole labelled in either the triazole or phenyl rings as a single oral dose at either 2 or 150 mg/kg bw, the radiolabel was rapidly and extensively (> 90%) absorbed from the gastrointestinal tract, the T_{max} calculated from plasma concentrations being 0.1–0.7 h for males and females. There were no significant differences related to sex, higher or lower dose or multiple doses.

The highest concentrations of radioactivity were found in the gastrointestinal tract and liver, as demonstrated by dissection and LSC and confirmed by whole-body autoradiography. Concentrations of radiolabel in the liver were markedly higher in male rats than in females. Relatively high concentrations were also found in the thyroid. Distribution was rapid and was followed by extensive loss of radioactivity from tissues and organs. The highest concentrations of prothioconazole equivalents were recorded in the liver, followed by kidney, fat, thyroid and adrenal gland.

Excretion was initially extensive and relatively rapid, mainly via the faeces, about > 70% being eliminated within 24 h, although the subsequent rate of excretion was low. Extensive biliary excretion (90%) was shown in bile-duct cannulated rats; evidence for enterohepatic recirculation was also seen in these rats.

Studies of metabolism using both phenyl- and triazole-ring-labelled molecules indicated that the prothioconazole structural skeleton remained largely intact, although prothioconazole was extensively metabolized. The major types of metabolic reactions identified were conjugation with glucuronic acid, oxidative hydroxylation of the phenyl moiety and desulfuration. The principal metabolites found in the excreta were prothioconazole-*S*-glucuronide, prothioconazole-desthio and prothioconazole itself. Many of the 18 metabolites identified were derived from the desthio metabolite (i.e. in which the triazole sulfur had been eliminated). The desthio metabolite was found almost exclusively in the faeces and represented between 3.5% and 17.7% of the administered dose. The systemic proportion of radiolabel as prothioconazole-desthio was very low; not more than about 0.07% of the administered dose was found in the urine. The *S*- or *O*-glucuronide conjugates were the principle systemic metabolites and were found in amounts of up to 7.7% of the administered dose in rat urine. These conjugates were also overall the most abundant, occurring at about 46% of the administered dose in bile, followed by the parent compound, prothioconazole (about 1–22%), and prothioconazole-desthio (about 0.4–18%).

Toxicological data

The acute toxicity of prothioconazole is low, the oral LD_{50} being > 6200 mg/kg bw in rats. At this dose, there were no deaths and clinical signs were limited to decreased motility and diarrhoea 1–6 h after dosing. The dermal LD_{50} in rats was > 2000 mg/kg bw and the inhalation LC_{50}, also in

rats, was > 4.9 mg/l for a 4-h exposure. Prothioconazole is not irritating to rabbit skin and eyes and is not sensitizing either in the Buehler skin patch test in guinea-pigs or in the local lymph node assay in mice.

Initial studies with repeated doses showed that prothioconazole could be unstable when formulated with diet, hence most studies were performed using dosing by gavage. A 4-week study in rats given prothioconazole by different dosing routes established that plasma concentrations in rats dosed by gavage at 1000 mg/kg bw per day were 3–6-fold those in rats given diets containing prothioconazole at 10 000 ppm, equivalent to 1000 mg/kg bw per day, and this was consistent with the observation of more marked effects in rats dosed by gavage.

The liver was consistently identified as a target organ in short-term studies in rats, mice and dogs, although there were some species differences in the hepatic effects observed. Increased liver weights and increased activities of several liver enzymes were observed in mice, rats (particularly females) and dogs. Microscopic lesions were also observed in the liver, including an increase in pigmented material in dogs, centrilobular fatty change and focal necrosis in mice and cytoplasmic changes and centrilobular hepatocellular hypertrophy in rats and mice. Some of these effects were consistent with induction of hepatic enzymes. None of the effects recorded in the liver persisted after 4- and 8-week recovery periods in rats and dogs, respectively.

The kidney was the primary target organ in dogs and was also identified as a target organ in rats, but not in mice. The effects on the kidneys consisted of increased weights and changes in histology, namely increased incidence and severity of basophilic tubules and tubular dilatation in rats, and interstitial fibrosis and inflammation in dogs. These findings did not persist after a recovery period in rats, but there was only partial recovery in dogs. In rats, these kidney changes correlated with greatly increased water intakes, indicating disturbance of kidney function and systemic water homeostasis.

The following NOAELs were derived from short-term studies in which prothioconazole was administered orally:

— In a 14-week study in mice dosed by gavage, the NOAEL was 25 mg/kg bw per day on the basis of increased liver weights and various histological changes in the liver at 100 mg/kg bw per day;

— In studies of up to 14 weeks in rats dosed by gavage, the NOAEL was 100 mg/kg bw per day on the basis of increased water consumption, decreased urine output, increased liver weights in females, and histological changes in the liver and kidney at 500 mg/kg bw per day;

— In 13-week and 1-year studies in dogs dosed by gavage, the overall NOAEL was 25 mg/kg bw per day on the basis of minimal histological changes in the kidneys at 40 mg/kg bw per day.

In long-term studies in rats and mice dosed by gavage, the primary target organs were the liver and kidney. There was no evidence for any carcinogenic potential in rats or mice. The hepatic effects observed in rats were increased incidences of eosinophilic or clear-cell foci. The other liver effects observed in rats and mice (increased weights, centrilobular hypertrophy with cytoplasmic changes) were consistent with induction of hepatic enzymes. There was slight alteration in the concentrations of plasma thyroid hormones in rats, but there was no associated thyroid histopathology.

The kidney effects in rats were increased organ weight and increased severity of chronic progressive nephropathy accompanied by markedly increased water consumption, effects on urine analysis, crystalline material in the urine sediment and transitional-cell hyperplasia in the urinary bladder. In mice, responses comprised decreased organ weight, tubular degeneration and regeneration and subcapsular tubular degeneration with interstitial fibrosis. The kidney effects were more marked in rats than in mice, and treatment of rats for more than 1 year was associated with prolonged and increasingly severe functional deficit in the kidneys. Males were consistently more markedly affected

than females. In rats, the kidney dysfunction and resulting dehydration caused mortality at doses of between 500 and 1000 mg/kg bw per day.

In a long-term study in rats dosed by gavage for 2 years, the NOAEL was 5 mg/kg bw per day on the basis of gross and microscopic changes in the liver and kidneys at 50 mg/kg bw per day. In a long-term study in mice dosed by gavage for 18 months, the NOAEL was 10 mg/kg bw per day on the basis of reduced body weights and gross and microscopic changes in the liver and kidneys at 70 mg/kg bw per day.

Prothioconazole was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. Although genotoxicity was not observed in tests for gene mutation in vitro, there was equivocal evidence for DNA damage and confirmed evidence for the induction of chromosomal aberrations in vitro; however, these observations were not confirmed in the relevant assays conducted in vivo.

The Meeting concluded that prothioconazole is unlikely to be genotoxic.

On the basis of the absence of carcinogenicity in rodents and the absence of genotoxicity in vivo, the Meeting concluded that prothioconazole is unlikely to pose a carcinogenic risk to humans.

In a multigeneration study in rats, effects were observed on the liver and kidneys at higher doses in parental animals. Some of these observations were consistent with the findings of short-term and long-term studies of toxicity. The NOAEL for systemic toxicity in the parental rats was 9.7 mg/kg bw per day on the basis of reduced body weight and effects on organ weights at 95.6 mg/kg bw per day. In the offspring, the NOAEL was 95.6 mg/kg bw per day on the basis of reduced pup-weight gain, reduced spleen weight and delayed preputial separation at 726 mg/kg bw per day. The NOAEL for reproductive effects was 95.6 mg/kg bw per day on the basis of disruption to the estrous cycle, reduced number of implantation sites and litter size, increased time to insemination and increased duration of gestation at 726 mg/kg bw per day. Although several of these observations were not statistically significantly different, they were consistent features of the group receiving the highest dose in contrast to the other groups, but they did not result in effects on mating, fertility or gestation indices.

The highest dose of 726 mg/kg bw per day caused parental toxicity that was probably related to kidney dysfunction and resulting dehydration, which in other studies in rats given repeated doses were a cause of mortality at doses of between 500 and 1000 mg/kg bw per day. Effects on developing pups also were restricted to the group receiving a dose of 726 mg/kg bw per day.

The Meeting concluded that prothioconazole was toxic to the reproductive system and to developing offspring at a dose that was accompanied by toxicity in parental rats.

In a study of developmental toxicity in which rats were given prothioconazole by gavage on days 6–19 of gestation, the NOAEL for maternal toxicity was 80 mg/kg bw per day on the basis of reduced body-weight gain and increased water consumption and urination at 500 mg/kg bw per day. Examination of the fetuses revealed an increased incidence of microphthalmia and of rudimentary supernumerary ribs, together with retarded fetal development, at 1000 mg/kg bw per day. Marked maternal toxicity was also recorded at this dose. Although the developmental effects, which included a statistically significant increased incidence in microphthalmia (on a fetal basis) occurred at a dose of 1000 mg/kg bw per day, microphthalmia was also observed in the groups at 500 and 80 mg/kg bw per day, but not in the controls. Rudimentary supernumerary ribs, which occur spontaneously in untreated rats of this strain, were significantly increased in a dose-related manner at all doses, including 80 mg/kg bw per day, the lowest dose tested. The Meeting noted there were indications that the incidence of this variation in the group receiving the vehicle only may have been particularly low in this experiment; however, incidences in all groups treated with prothioconazole were higher than the upper limit of the range for historical controls over the relevant period.

In order to further investigate the occurrence of microphthalmia, a different rat substrain was selected for which the available database on historical controls revealed a virtually-zero background

incidence of this malformation. Since the strain was nevertheless sensitive to direct, specific oculoteratogenic effects, it was well suited for investigation of the specificity of microphthalmia formation caused by prothioconazole. In this second study of developmental toxicity in rats, prothioconazole did not cause microphthalmia or other specific malformations at any dose up to and including 750 mg/kg bw per day. These results would seem to support the hypothesis that the increase in microphthalmia seen in the original study of developmental toxicity was a non-specific enhancement of a common spontaneous effect; however, the mechanism by which microphthalmia was induced has not been investigated or described. There was an increase in the incidence of rudimentary (comma shaped) supernumerary 14th ribs that was significant on a fetal basis at 750 mg/kg bw per day, but not on a litter basis, and was not increased at 80 mg/kg bw per day or at the lowest dose. The NOAEL for maternal toxicity was 80 mg/kg bw per day on the basis of reduced body-weight gain, increased water consumption, reduced food consumption and clinical chemical indications for functional impairment of liver and kidney function at 750 mg/kg bw per day. The NOAEL for developmental toxicity was 80 mg/kg bw per day on the basis of a statistically significant increase in the incidence of rudimentary supernumerary 14th ribs at 726 mg/kg bw per day.

In a study of developmental toxicity in which rabbits were given prothioconazole by gavage on days 6–27 of gestation, the NOAEL for maternal toxicity was 80 mg/kg bw per day on the basis of mortality, body-weight loss or reduced body-weight gain and reduced food consumption at 350 mg/kg bw per day. The NOAEL for developmental toxicity was 80 mg/kg bw per day on the basis of abortions, total litter losses, reduced fetal weights and retarded ossification at 350 mg/kg bw, where there was clear evidence of severe maternal toxicity.

In a study of neurotoxicity in rats given a single dose of prothioconazole by gavage, the NOAEL was 218 mg/kg bw per day on the basis of transient clinical signs at 877 mg/kg bw per day. There were no neurohistopathological changes in nerve tissue and no persistent signs of neurobehavioural toxicity.

In a 90-day study of neurotoxicity in rats given prothioconazole by gavage, the NOAEL was 100 mg/kg bw per day on the basis of clinical signs, reduced body weights and reduced motor and locomotor activity at 1000 mg/kg bw per day. The reduced motor and locomotor activity is likely to be secondary to the systemic toxicity evident in these animals rather than clear neurobehavioural toxicity. There were no neurohistopathological changes in nerve tissue or muscle.

The Meeting concluded that prothioconazole is unlikely to cause neurotoxicity in humans.

There were no indications of immunotoxicity in general studies of toxicity in dogs, rats and mice.

Some aspects of the toxicology of certain metabolites of prothioconazole found in wheat (mainly straw), but not necessarily in rats—exceptions being prothioconazole-triazolinone (M03) and prothioconazole-desthio—were investigated.

Triazole (1,2,4-triazole) and its metabolites, triazole alanine and triazole acetic acid, are metabolites of difenoconazole, the toxicology of which was summarized by JMPR 2007 and by the present Meeting. No triazole-free metabolites were found using phenyl-labelled prothioconazole. The other metabolites summarized here were prothioconazole-desthio, prothioconazole-sulfonic acid (M02), prothioconazole-desthio-*alpha*-hydroxy (M18), prothioconazole-desthio-*alpha*-acetoxy (M19), prothioconazole-benzylpropyldiol (M09) and prothioconazole-triazolinone (M03). M03 is also found in rat urine in which it represents up to 2% of the administered parent compound. The data submitted on these substances indicate that, except for prothioconazole-desthio and M02, they are not toxicologically relevant metabolites. A single-dose study of oral toxicity indicated that the LD_{50} of prothioconazole-sulfonic acid is > 200 mg/kg bw and < 2000 mg/kg bw. The LD_{50} values for prothioconazole-desthio-*alpha*-hydroxy (M18), prothioconazole-desthio-*alpha*-acetoxy (M19) and prothioconazole-benzylpropyldiol are all > 2000 mg/kg bw. Prothioconazole-sulfonic acid has been tested in a 90-day dietary study of toxicity in rats. The NOAEL was 500 ppm, equal to 34 mg/kg bw per day,

on the basis of histomorphological alterations in the urinary bladder at 2000 ppm, equal to 136 mg/kg bw per day. No other repeat-dose studies of toxicity have been conducted with these metabolites.

In a study of developmental toxicity in rats given prothioconazole-sulfonic acid by gavage on days 6–20 of gestation, the NOAEL for maternal toxicity was 150 mg/kg bw per day on the basis of increased mortality, reduced food consumption and reduced body-weight gain at 750 mg/kg bw per day. The NOAEL for developmental toxicity was 150 mg/kg bw per day on the basis of increased incidence of total implantation loss and the occurrence of reduced fetal weight gain and reduced ossification at 750 mg/kg bw per day. Prothioconazole-sulfonic acid did not show any teratogenic potential.

None of the metabolites was active in tests for mutagenicity with strains of *S. typhimurium*.

Prothioconazole-desthio

The most toxicologically significant of the prothioconazole metabolites is prothioconazole-desthio. A largely complete toxicology dossier was available for this compound.

Prothioconazole-desthio was rapidly and almost completely absorbed from the gastrointestinal tract of rats, with a plasma T_{max} of about 1.5 h, but maximum plasma concentrations were low. The plasma T_{max} of prothioconazole-desthio in pregnant rats treated by gavage was similar to that in male rats. The mean concentration of radioactivity in the body minus the gastrointestinal tract was < 3.5% of the administered dose, indicating that there was little distribution to the peripheral tissues; the highest concentrations (about 3% of the administered dose) were found in the liver. Excretion occurred predominantly via the bile, and the elimination half-life and mean residence time were prolonged due to intensive enterohepatic recirculation. No potential for bioaccumulation was expected. The bile metabolites identified indicated that metabolism proceeded via oxidation only of the phenyl moiety, with subsequent glucuronidation and methylation of the oxidation products. These oxidation reactions yielded metabolites without their former aromatic character; nevertheless, the cyclopropyl and triazole ring structures of prothioconazole-desthio remained intact.

The acute toxicity of prothioconazole-desthio is low, the oral LD_{50} being approximately 2200 mg/kg bw in rats and mice. In both species, deaths were delayed, by 4–13 days in rats and 1–4 days in mice. In rats and mice, no clinical signs were observed at 100 mg/kg bw. The observations recorded for mice given higher doses were apathy, piloerection, laboured breathing, staggering gait and increased urination in males at 500 mg/kg bw and in females at 1000 mg/kg bw. Spastic gait and reduced mobility were also noted in females at > 100 mg/kg bw, but these signs were noted in males only at doses of > 2000 mg/kg bw. Atony, weak reflexes, emaciation, pallor, narrowed palpebral fissures (separation between the upper and lower eyelids), red crusted eyelids, bloody snout, prone position and leg extension occurred in males and females at high doses. Some clinical signs were evident shortly after treatment but others showed a delayed onset. All signs had resolved by day 13 in male rats and by day 18 in females. In mice, the clinical signs of response were motility and respiratory disturbances, piloerection, staggering gait, narrowed palpebral fissures, lacrimation, a spasmodic state, temporary rolling over, prostration or lying on the side These were mainly observed at up to moderate intensity, developed shortly after treatment in some cases, and persisted at maximum levels up to the eleventh day of the study in the male mice or up to the seventh day in the females. The dermal LD_{50} in rats was > 5000 mg/kg bw and the inhalation LC_{50}, also in rats, was > 5.08 mg/l for an exposure of 4 h. Prothioconazole-desthio is not irritating to rabbit skin and eyes and is not sensitizing in the Buehler skin patch test in guinea-pigs.

In short-term studies of toxicity, a common target organ in rat, mouse and dog was the liver and effects on this organ formed the basis for the NOAEL in the short-term studies in rats. Effects in the liver (not always adverse and not always at critical doses for those effects that were adverse) included increased organ weight, induction of CYP isoenzymes, hepatocellular hypertrophy, increased hepato-

cytic fatty vacuolation, single-cell or focal necrosis, hydropic degeneration and increased ploidy. The NOAELs in dietary studies were 2.2 mg/kg bw per day in a 13-week study in rats and 10 mg/kg bw per day in a 30-week study in dogs. No NOAEL was identified in mice, but it was certainly greater than 12 mg/kg bw per day.

Long-term dietary studies in rats and mice of prothioconazole-desthio confirmed that the primary target organ was the liver. The liver effects were increased weights, hypertrophy, cytoplasmic change and a shift in fat storage from the periportal (usual) to the centrilobular region of the liver in rats and increased incidences of periacinar fat accumulation in the liver of mice. Mild alteration in plasma thyroid hormone concentrations in rats was possibly a consequence of induction of hepatic enzymes, but there was no accompanying notable histopathology in the thyroids. In addition, in rats, there were increased incidences of adrenal cortical vacuolization in males at either of the two highest doses. There was no evidence for carcinogenicity of prothioconazole-desthio in rats or mice. The NOAEL in a 2-year dietary study in rats was 20 ppm, equal to 1.1 mg/kg bw per day, on the basis of microscopic changes in the liver and ovary at 140 ppm, equal to 8.0 mg/kg bw per day. The NOAEL in a 2-year dietary study in mice was 12.5 ppm, equal to 3.1 mg/kg bw per day, on the basis of microscopic changes in the liver at 50 ppm, equal to 12.8 mg/kg bw per day.

Prothioconazole-desthio was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. Genotoxicity was not observed in any of these assays.

The Meeting concluded that prothioconazole-desthio is unlikely to be genotoxic.

On the basis of the absence of carcinogenicity in rodents and the absence of genotoxicity, the Meeting concluded that prothioconazole-desthio is unlikely to pose a carcinogenic risk to humans.

The reproductive toxicity of prothioconazole-desthio was investigated in a one-generation pilot study and a two-generation study of reproduction in rats. One study for potential developmental toxicity after oral dosing was conducted in rats and one in rabbits. In addition, prothioconazole-desthio was tested for developmental neurotoxicity in one study in rats. Reproductive effects of prothioconazole-desthio in rats comprised reduced litter size, reduced pup viability, pre-weaning growth retardation and an increased incidence of cleft palate. In the main two-generation study, a number of females in the parental and F_1 generations exhibited dystocia (difficulty in giving birth). In both the pilot and main study, the NOAELs for parental toxicity were similar to, or lower than, the NOAELs for reproductive and neonatal effects. The NOAEL for systemic toxicity in the parental rats was 40 ppm, equal to 2.7 mg/kg bw per day, on the basis of hepatocellular vacuolation in males at 160 ppm, equal to 10.4 mg/kg bw per day. In the offspring, the NOAEL was 160 ppm, equal to 10 mg/kg bw per day, on the basis of decreased neonatal viability, reduced pup-weight gain, and cleft palate at 640 ppm, equal to 41 mg/kg bw per day. The NOAEL for reproductive effects was 40 ppm, equal to 10 mg/kg bw per day, on the basis of dystocia at 640 ppm, equal to 41 mg/kg bw per day.

In a study of developmental toxicity in rats given prothioconazole-desthio by gavage on days 6–15 of gestation, the NOAEL for maternal toxicity was 30 mg/kg bw per day on the basis of reduced body-weight gain, reduced food consumption and increased liver weight and histological changes in the liver at 100 mg/kg bw per day. There was no NOAEL for developmental toxicity in this study, in which there were increased incidences of fetuses with supernumerary ribs at all doses, including 10 mg/kg bw per day, the lowest dose tested. In a follow-up to this study, another study of developmental toxicity was conducted in rats given prothioconazole-desthio over a lower dose range by gavage on days 6–15 of gestation. The NOAEL for developmental toxicity was 1 mg/kg bw per day on the basis of increased incidence of supernumerary rudimentary ribs at 3 mg/kg bw per day.

In a study of developmental toxicity in rabbits given prothioconazole-desthio by gavage on days 6–18 of gestation, the NOAEL for maternal toxicity was 2 mg/kg bw per day on the basis of histological changes in the liver at 10 mg/kg bw per day. The NOAEL for developmental toxicity was 2 mg/kg bw per day on the basis of increased incidence of fetuses with any abnormality (primarily arthrogryposis and cleft palate) at 10 mg/kg bw.

In a study of developmental neurotoxicity in rats given prothioconazole-desthio from day 6 of gestation until day 21 of lactation, the NOAEL was 500 ppm, equal to 43.3 mg/kg bw per day, the highest dose tested, on the basis of the absence of effects on neurobehavioural, learning and memory parameters, on brain weight, brain morphometry and on neuropathology parameters at this dose.

In summary, on the basis of the results of the submitted studies of toxicity, the acute oral toxicity of both prothioconazole and its desthio metabolite was low and neither compound showed any mutagenic or carcinogenic potential. The NOAELs for the short-term and long-term studies as well as the studies of reproductive toxicity and developmental toxicity were clearly lower for prothioconazole-desthio than for prothioconazole. In the studies of developmental toxicity, increased incidences of cleft palate in rats and rabbits were observed with prothioconazole-desthio at doses of 100 and 50 mg/kg bw per day, respectively, with no cleft palate induction at 30 and 10 mg/kg bw per day, respectively. Cleft palate was not observed in studies of developmental toxicity with the parent compound, prothioconazole, but was observed in a study of reproductive toxicity in rats given prothioconazole at a dose of 41 mg/kg bw per day.

No adverse effects have been identified in workers involved in the development, production or formulation of prothioconazole. No further information on medical surveillance or poisoning incidents was available.

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

Toxicological evaluation

Prothioconazole

An ADI of 0–0.05 mg/kg bw was established for prothioconazole based on the NOAEL of 5 mg/kg bw per day, identified on the basis of gross and microscopic changes in the liver and kidneys in a 2-year study of toxicity and carcinogenicity in rats treated by gavage, and a safety factor of 100.

An ARfD of 0.8 mg/kg bw was established for women of childbearing age based on a NOAEL of 80 mg/kg bw per day, identified on the basis of a marginally increased incidence of supernumerary rudimentary ribs that might be attributable to a single exposure at 750 mg/kg bw per day in a study of developmental toxicity in rats, and with a safety factor of 100. The Meeting concluded that the establishment of an ARfD for the general population was not necessary on the basis of its low acute toxicity, the lack of evidence for any acute neurotoxicity and absence of any other toxicologically relevant effect that might be attributable to a single dose.

Prothioconazole-desthio

Since the residue definition for risk assessment in all commodities is expressed as prothioconazole-desthio and this metabolite is of higher toxicity than the parent, ARfD values and an ADI were also established for prothioconazole-desthio.

An ADI of 0–0.01 mg/kg bw was established for prothioconazole-desthio based on the NOAEL of 1.1 mg/kg bw per day, identified on the basis of microscopic changes in the liver and ovaries in a 2-year dietary study of toxicity and carcinogenicity in rats, and with a safety factor of 100.

An ARfD of 0.01 mg/kg bw was established for women of childbearing age based on a NOAEL of 1 mg/kg bw per day, identified on the basis of increased incidence of supernumerary rudimentary ribs that might be attributable to a single exposure at 3 mg/kg bw per day in a study of developmental toxicity in rats, and with a safety factor of 100. Although the increased incidence at 3 mg/kg bw

per day was only significant on the basis of the number of fetuses, this was the lower limit of a clear dose-related response curve.

The Meeting also established an ARfD of 1 mg/kg bw for the general population based on a NOAEL of 100 mg/kg bw, identified on the basis of clinical signs in studies of toxicity in mice and rats given single doses, and a safety factor of 100.

Levels relevant to risk assessment for prothioconazole

Species	Study[a]	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity	Toxicity	10 mg/kg bw per day	70 mg/kg bw per day
		Carcinogenicity	500[b] mg/kg bw per day	—
Rat	Two-year studies of toxicity and carcinogenicity	Toxicity	5 mg/kg bw per day	50 mg/kg bw per day
		Carcinogenicity	750[b] mg/kg bw per day	—
	Two-generation study of reproductive toxicity	Reproductive toxicity	95.6 mg/kg bw per day	726 mg/kg bw per day
		Parental toxicity	9.7 mg/kg bw per day	95.6 mg/kg bw per day
		Offspring toxicity	95.6 mg/kg bw per day	726 mg/kg bw per day
	Developmental toxicity	Maternal toxicity	80 mg/kg bw per day	750 mg/kg bw per day
		Embryo and fetal toxicity	80 mg/kg bw per day	750 mg/kg bw per day
Rabbit	Developmental toxicity	Maternal toxicity	80 mg/kg bw per day	350 mg/kg bw per day
		Embryo and fetal toxicity	80 mg/kg bw per day	350 mg/kg bw per day

[a] In all cases, prothioconazole was administered by gavage.

[b] Highest dose tested.

Levels relevant to risk assessment for prothioconazole-desthio

Species	Study	Effect	NOAEL	LOAEL
Mouse	Single dose LD_{50}	Toxicity	100 mg/kg bw	500 mg/kg bw
	Two-year study of toxicity and carcinogenicity	Toxicity	12.5 ppm, equal to 3.1 mg/kg bw per day	50 ppm, equal to 12.8 mg/kg bw per day
		Carcinogenicity	200 ppm, equal to 51.7 mg/kg bw per day[b]	—

Rat	Single-dose LD_{50} study	Toxicity	100 mg/kg bw	500 mg/kg bw
	Two-year studies of toxicity and carcinogenicity	Toxicity	20 ppm equal to 1.1 mg/kg bw per day	140 ppm equal to 8.0 mg/kg bw per day
		Carcinogenicity	980 ppm equal to 57.6[b] mg/kg bw per day	—
	Two-generation study of reproductive toxicity	Reproductive toxicity	160 ppm equal to 10.0 mg/kg bw per day	640 ppm equal to 41.2 mg/kg bw per day
		Parental toxicity	40 ppm equal to 2.7 mg/kg bw per day	160 ppm equal to 10.4 mg/kg bw per day
		Offspring toxicity	160 ppm equal to 10.0 mg/kg bw per day	640 ppm equal to 41.2 mg/kg bw per day
	Developmental toxicity	Maternal toxicity	30 mg/kg bw per day[b]	—
		Embryo and fetal toxicity	1 mg/kg bw per day	3 mg/kg bw per day
Rabbit	Developmental toxicity	Maternal toxicity	2 mg/kg bw per day	10 mg/kg bw per day
		Embryo and fetal toxicity	2 mg/kg bw per day	10 mg/kg bw per day
Dog	Thirty-week study of toxicity	Toxicity	10.1 mg/kg bw per day	69.9 mg/kg bw per day

[b] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.05 mg/kg bw (for prothioconazole)

0–0.01 mg/kg bw (for prothioconazole-desthio)

Estimates of acute reference doses

0.8 mg/kg bw for women of childbearing age (for prothioconazole)

Unnecessary for the general population (for prothioconazole)

0.01 mg/kg bw for women of childbearing age (for prothioconazole-desthio)

1 mg/kg bw for the general population (for prothioconazole-desthio).

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 prothioconazole and prothioconazole-desthio

Absorption, distribution, excretion and metabolism in mammals		
Rate and extent of oral absorption	Rapid and extensively, > 90%	
Distribution	Distributed throughout the body; higher concentrations in liver and gastrointestinal tract	
Potential for accumulation	No evidence	
Rate and extent of excretion	High, > 70% within 24 h, but subsequently low rate	
Metabolism in animals	18 metabolites identified	
Toxicologically significant compounds (animals, plants and environment)	Parent, prothioconazole-desthio and M02	
Acute toxicity	*Prothioconazole*	*Prothioconazole-desthio*

Rat, LD_{50}, oral	> 6200 mg/kg bw	> 2500 mg/kg bw
Rat, LC_{50}, inhalation	> 4.9 mg/l[a] (4 h)	> 5.08 mg/l[a] (4 h)
Rat, LD_{50}, dermal	> 2000 mg/kg bw[a]	> 5000 mg/kg bw[a]
Rabbit, dermal irritation	Not irritating	Not irritating
Rabbit, ocular irritation	Not irritating	Not irritating
Dermal sensitization	Not sensitizing (Buehler skin patch test in guinea-pigs; lymph node assay in mice)	Not sensitizing (Buehler skin patch test in guinea-pigs)
Short-term studies of toxicity		
Target/critical effect	Liver, kidney	Liver
Lowest relevant oral NOAEL	25 mg/kg bw per day (3-month study in dogs,	2.2 mg/kg bw per day (13-week study in rats
Lowest relevant dermal NOAEL	1000 mg/kg bw per day[a] (4-week study in rats)	No data
Lowest relevant inhalation NOAEC	No data	No data
Genotoxicity		
	Not genotoxic in vivo, but mixed results in vitro	Not genotoxic
Long-term studies of toxicity and carcinogenicity		
Target/critical effect	Liver, kidney	Liver, kidney
Lowest relevant NOAEL	5 mg/kg bw per day (2-year study in rats)	1 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic	Not carcinogenic
Reproductive toxicity		
Reproductive target/critical effect	Disruption of estrous cycle; reduced implantation sites and litter size; increased time to insemination; increased gestation time; all associated with severe maternal toxicity	Decreased neonatal viability, reduced pup-weight gain, and cleft palate at maternal toxic doses
Lowest relevant reproductive NOAEL	100 mg/kg bw per day	10 mg/kg bw per day
Developmental target/critical effect	Not teratogenic; abortions, total litter loss, reduced fetal body weight, delayed ossifications, rudimentary supernumerary ribs	Teratogenic (cleft palate, arthrogryposis); abnormal fetuses, increased incidence of supernumerary rudimentary ribs
Lowest relevant developmental NOAEL	80 mg/kg bw per day (rat, rabbit)	1 mg/kg bw per day (rat, rabbit)
Neurotoxicity/delayed neurotoxicity		
	No signs of neurotoxicity	No signs of neurotoxicity
Other toxicological studies		
	Induction of liver xenobiotic metabolizing enzymes	Clinical signs of toxicity in single-dose studies (LD_{50}) in rats and mice

		Several metabolites in addition to prothioconazole-desthio and MO2 have been investigated, but are not considered to be toxicologically significant	
Medical data			
		No reports of toxicity in workers exposed during manufacture or use	
Summary			
Prothioconazole	Value	Study	Safety factor
ADI	0–0.05 mg/kg bw	Dog, 1-year study of toxicity; and rat, 2-year study of toxicity and carcinogenicity	100
ARfD	0.8 mg/kg bw for women of childbearing age	Rat, study of developmental toxicity	100
Prothioconazole-desthio	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Rat, 2-year study of toxicity and carcinogenicity	100
ARfD	0.01 mg/kg bw for women of childbearing age	Rat, study of developmental toxicity	100
	1 mg/kg bw for the general population	Rat and mouse, LD_{50} studies	100

[a] Only dose tested.

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SPINETORAM

First draft prepared by
F. Metruccio[1] & A. Boobis[2]

[1]International Centre for Pesticides & Health Risk Prevention, Milan, Italy; and
[2]Experimental Medicine & Toxicology Division of Investigative Science,
Faculty of Medicine, Imperial College, London, England

Explanation

Spinetoram, also known as XDE-175 or XR-175, is a fermentation product derived from the actinomycete bacterium *Saccharopolyspora spinosa,* which has been slightly modified by chemical reaction. Spinetoram is a macrocyclic lactone insecticide. It acts by causing persistent activation of insect nicotinic acetylcholine receptors.

Spinetoram is composed of numerous spinosyns, known as "factors", which differ slightly from each other. Each spinosyn consists of a large complex hydrophobic ring, a basic amine group, and two sugar moieties. The insecticidal activity of spinetoram is attributed to two spinosyns, identified

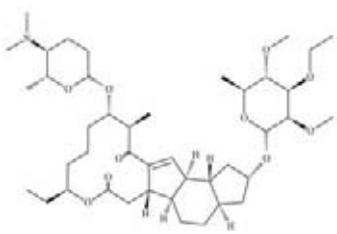
as XDE-175-J ("factor J") and XDE-175-L ("factor L"), which comprise the overwhelming majority of the technical material (Figure 1). The ratio of factor J to factor L ranges from 70 : 30 to 90 : 10. Unless otherwise stated, the studies of toxicity described in the present monograph were conducted with factor J and factor L in a ratio equal to 75 : 25. Some studies were repeated with factor J and factor L in the ratio of 85 : 15; this was done to demonstrate that the 85 : 15 ratio produces a toxicity profile that is essentially the same as that seen with a ratio of 75 : 25 ratio.]

The remaining components of spinetoram comprise a number of additional spinosyns (that have minor substitutions at various locations in the spinosyn molecule) and other impurities consisting of inorganic salts, carbohydrates and proteinaceous material that would be expected to be produced during a fermentation process.

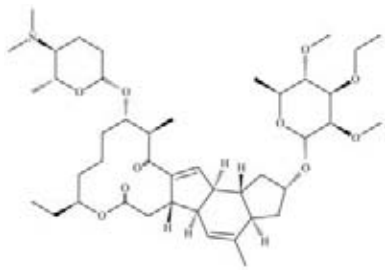
Spinetoram has not been evaluated previously by the JMPR and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide residues (CCPR).

All the pivotal studies met the basic requirements of the relevant (Organization for Economic Co-operation and Development) OECD or national test guidelines and included certificates of compliance with good laboratory practice (GLP).

Figure 1. Chemical structures of the two principal spinosyns (factors J and L) contained in spinetoram



XDE-175-J (factor J)



XDE-175-L (factor L)

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

The two insecticidally active factors in spinetoram, identified as spinosyns XDE-175-J and XDE-175-L (factors J and L), were evaluated separately in a series of studies of absorption, distribution and metabolism. Groups of four male and female Fischer 344 rats were given a single dose of macrolide-ring ^{14}C-labeled factor at 10 or 100 mg/kg bw by oral gavage; unlabelled compound as repeated oral doses at 10 mg/kg bw per day for 14 days, followed by a single radiolabelled dose; or an intravenous dose of 10 mg/kg bw of each of the two active factors.

After dosing by oral gavage, both factor J and factor L were rapidly absorbed without any apparent lag time. With factor J, peak plasma concentrations of radiolabel were attained within approximately 2 h in males and females at both doses, while with factor L, peak plasma concentrations of radiolabel were seen at 2 h in females and 4 h in males, at both doses. Plasma elimination half-lives of radiolabel ranged from 4 h to 24 h depending on dose and factor, with the radiolabel clearing slightly more slowly with factor L than with factor J. Comparison of plasma area-under-the-curve of concentration–time (AUCs) of radiolabel from rats dosed orally with those from rats dosed intra-

venously indicated that a minimum of 26–29% of factor J and 39–57% of factor L was systemically available after the lower dose (10 mg/kg bw), with higher amounts (37–36% factor J and 73–92% factor L) systemically available after a higher dose (100 mg/kg bw). Based on data on excretion of metabolites in the urine and faeces (Tables 1 and 2), it was estimated that at least 70% of an oral dose was absorbed, possibly with some pre-systemic metabolism. However, no studies were conducted in bile-duct cannulated rats. AUCs after oral administration were slightly greater than proportional to dose: 14-fold for ZDE-175-J and 20-fold for factor J. The reason for this was not clear.

The radiolabel remaining in the tissues and carcass 7 days after dosing was approximately 0.6–1.4% of the administered dose of factor J regardless of dose, and approximately 3% of factor L at the lower dose (10 mg/kg bw) and 7% at the higher dose (100 mg/kg bw). At termination (168 h after dosing), there were no differences in tissue concentrations of radiolabel between rats given a single oral dose of radiolabelled material or repeated doses of unlabelled material followed by a single radiolabelled dose of either factor J or factor L.

With factor J, the concentration of ^{14}C residues in tissues collected at T_{max} generally decreased in relative order from the gastrointestinal tract > lymph nodes > liver > lungs > adrenals > spleen. The rank order of residue concentrations in tissues of rats killed at $t = ½T_{max}$ remained approximately the same for the gastrointestinal tract, lymph nodes, lungs, adrenals, and spleen; however, the rank order of liver residues was lower, while the rank order for residues in fat and bone marrow was higher. Concentrations of factor J and related metabolites were observed in the liver, kidney, plasma, and thyroid tissue extracts of male and female rats given this compound orally. The parent compound was detected in all four tissues chemically analysed from the T_{max} groups and in all except plasma from the $t = ½T_{max}$ groups. Parent factor J was most abundant in the liver, with concentrations at T_{max} ranging from 1.4% to 3.1% of the administered radiolabel.

With factor L, the concentration of ^{14}C residues in tissues collected at T_{max} generally decreased in relative order from the gastrointestinal tract > lymph nodes > liver > lungs > adrenals > spleen. The rank order of residue concentrations in tissues of rats killed at $t = ½T_{max}$ remained approximately the same for the gastrointestinal tract, lymph nodes, adrenals, and lungs; however, the rank order of liver residues was lower, while the rank order of residues in fat and bone marrow was higher. Concentrations of factor L test material and related metabolites were observed in the liver, kidney, plasma, and thyroid tissue extracts of male and female rats given this compound orally. Parent compound was detected in all four tissues chemically analysed from the T_{max} group and the $t = ½T_{max}$ group. Parent factor L was most abundant in the liver, with concentrations at T_{max} ranging from 3.4% to 6.0% of the administered radiolabel.

Although tissue concentrations indicated very low residues of spinetoram, studies of repeated dietary exposure revealed a toxicity profile that was consistent with the slow accumulation of very small quantities of spinetoram during prolonged exposure. This toxicity profile is consistent with trapping of a cationic amphiphilic compound within the lysosomes. These effects have been shown to be reversible upon cessation of exposure.

Both factor J and factor L were highly metabolized in male and female rats given these compounds orally or intravenously. Parent compound accounted for 7–40% (factor J) and 7–26% (factor L) of the total radiolabel eliminated in the faeces. Most of the radiolabel in the urine and in faecal extracts was present as seven or nine metabolites (for factor J or factor L respectively). One major metabolite (the cysteine conjugate of factor J and factor L) was present in all urine and faecal extracts and accounted for 31–61% and 51–66% of the administered dose (for factor J or factor L, respectively).

The major metabolic pathway was via glutathione conjugation of the parent, and glutathione conjugation of metabolites arising from *N*-demethylation and *O*-deethylation of each factor, as well as hydroxylation and deglycosylation of parent factor J. In addition, small amounts of the sulfate and glucuronide conjugates of the aglycone of factor L were detected. The proposed pathways for metabolism of factor J and factor L are shown in Figures 2 and 3.

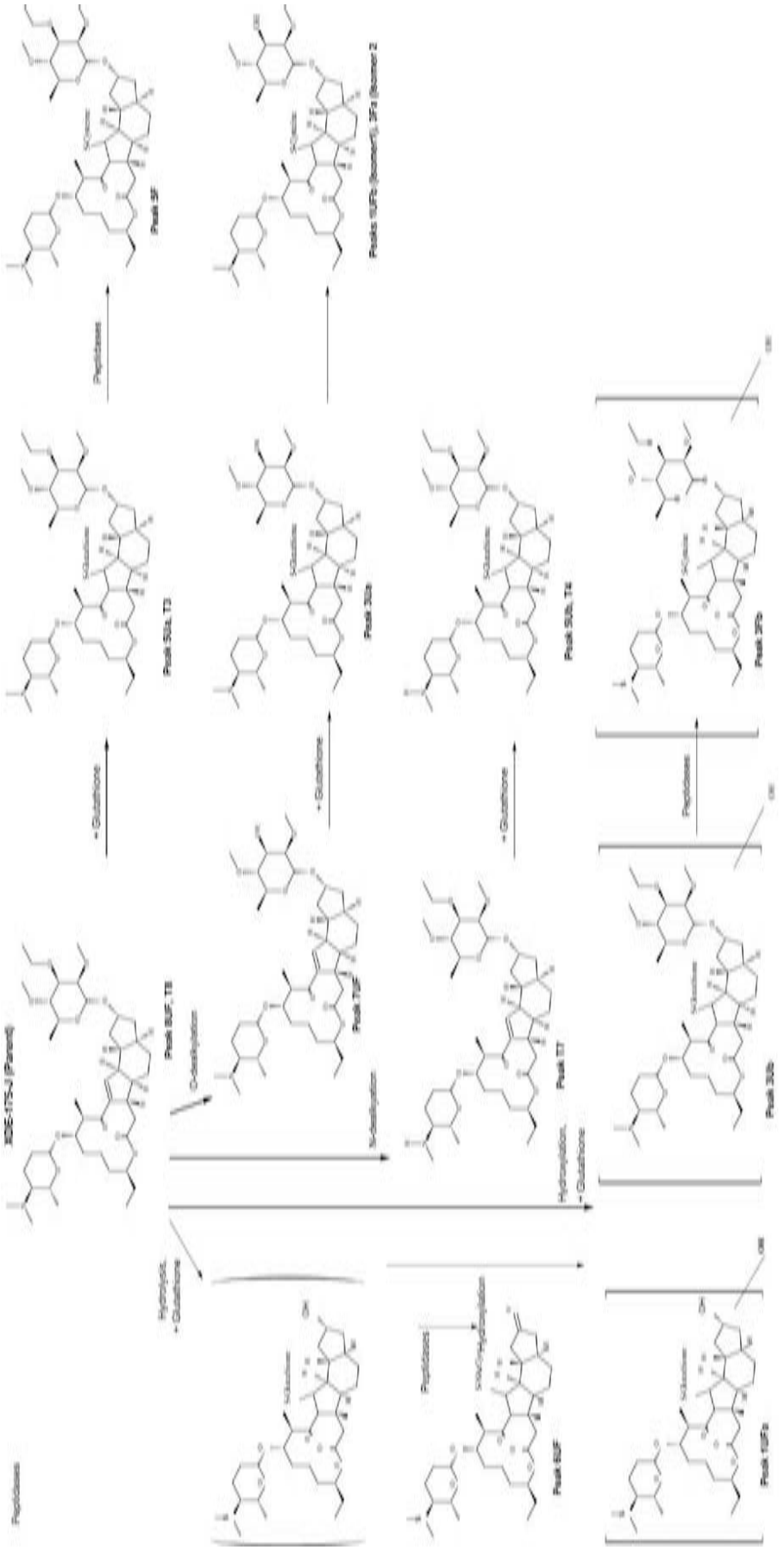


Figure 2. Proposed pathway for metabolism of factor J

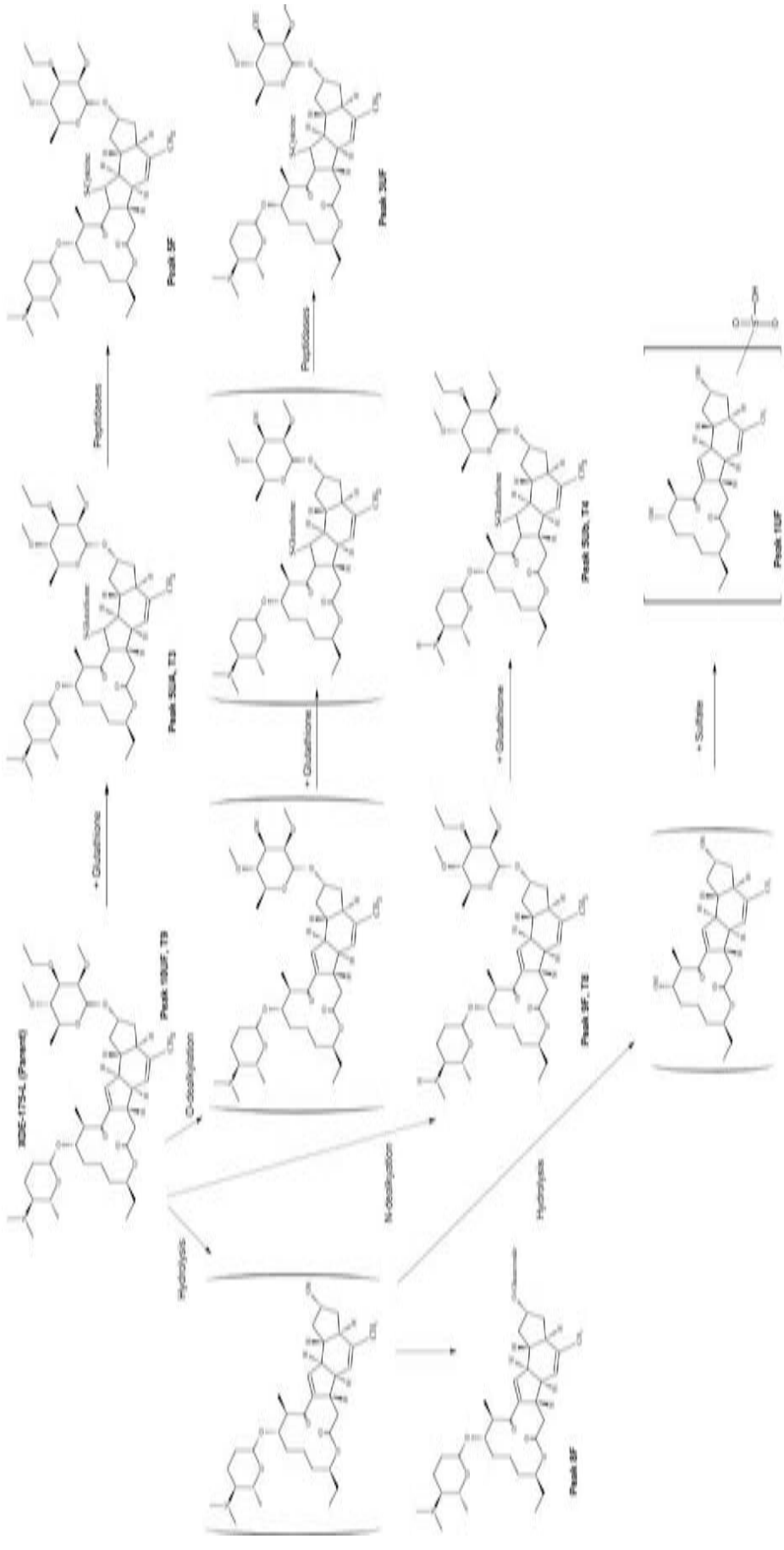


Figure 3. Proposed pathway for metabolism of factor L

Table 1. Recovery of radiolabel in tissues and excreta of rats given radiolabelled spinosyn factor J

Sample	Recovery of radiolabel (% of administered dose)							
	Single dose at 10 mg/kg bw, by gavage		Single dose at 100 mg/kg bw, by gavage		Pre-treatment followed by single dose at 10 mg/kg bw, by gavage[a]		Single dose at 10 mg/kg bw, intravenous	
	Males	Females	Males	Females	Males	Females	Males	Females
Expired air	NQ	NQ	NA	NA	NA	NA	NA	NA
Tissues	0.30	0.38	0.44	0.81	0.28	0.32	0.89	0.84
Carcass	0.19	0.40	0.55	1.010.17	0.17	0.41	0.61	1.30
Cage wash	0.73	0.01	0.11	0.11	0.42	0.04	0.14	0.25
Urine:								
0–12 h	2.90	3.48	2.53	2.73	2.23	2.71	6.43	6.29
12–24 h	0.50	0.58	0.75	0.79	0.41	0.65	0.93	1.12
24–48 h	0.25	0.28	0.34	0.56	0.28	0.40	0.88	0.85
48–72 h	0.16	0.05	0.22	0.21	0.15	0.12	0.34	0.55
72–96 h	0.13	0.07	0.13	0.24	0.11	0.07	0.18	0.41
96–120 h	0.04	0.05	0.06	0.09	0.06	0.05	0.11	0.17
120–144 h	0.03	0.03	0.04	0.06	0.03	0.05	0.08	0.11
144–168 h	0.02	0.03	0.11	0.05	0.02	0.02	0.05	0.09
Faeces:								
0–24 h	78.39	69.32	70.16	57.75	76.94	74.96	52.49	59.31
24–48 h	5.27	11.01	8.51	18.57	6.00	10.03	14.46	13.52
48–72 h	1.72	2.24	2.24	3.43	1.55	2.32	5.87	5.34
72–96 h	0.73	0.93	1.24	1.89	0.67	1.00	2.21	3.00
96–120 h	0.38	0.53	0.63	1.04	0.30	0.64	1.16	2.02
120–144 h	0.22	0.33	0.47	0.72	0.21	0.38	0.74	1.08
144–168 h	0.13	0.21	0.48	0.54	0.17	0.25	0.51	0.80
Total	92.10	89.93	89.01	90.60	90.00	94.42	88.08	97.05

From Rick et al. (2005a)
NA, not analysed; NQ, not quantifiable; radiolabel was not detected in tissues at a concentration exceeding the limit of quantitation (LOQ).
[a] Unlabelled compound as repeated oral doses at 10 mg/kg bw per day for 14 days, followed by a single radiolabelled dose.

Rates of faecal and urinary elimination of factor J and factor L were similar, regardless of dose, sex, number of doses, or route of administration. Faeces represented the primary route of excretion. On average, approximately 85% of the administered dose was excreted in the faeces, with most being excreted in the first 24 h. Urine accounted for approximately 3–4% of the administered dose. The half-lives of faecal excretion were approximately 24 h, with urinary excretion half-lives of approximately 24–30 h (Rick et al., 2005a, 2005b, 2007a, 2007b).

1.2 Bioavailability

Mice

In a study of toxicity (also described in section 2.2) in CD-1 mice given diets containing spinetoram (purity, 95%; ratio, 64% factor J and 31% factor L) at doses ranging from approximately 8

Table 2. Recovery of radiolabel in tissues and excreta of rats given radiolabelled spinosyn factor L

Sample	Recovery of radiolabel (% of administered dose)							
	Single dose at 10 mg/kg bw, by gavage		Single dose at 100 mg/kg bw, by gavage		Pre-treatment followed by single dose at 10 mg/kg bw, by gavage [a]		Single dose at 10 mg/kg bw, intravenous	
	Males	Females	Males	Females	Males	Females	Males	Females
Expired air	NQ	NQ	NA	NA	NA	NA	NA	NA
Tissues	1.23	2.22	4.39	3.63	1.98	1.17	3.49	7.08
Carcass	1.46	1.70	2.74	3.15	1.32	1.65	3.69	5.75
Cage wash	0.46	0.08	0.06	0.19	0.41	0.04	0.58	0.08
Urine:								
0–12 h	1.71	1.75	1.54	1.55	1.59	1.47	2.43	2.19
12–24 h	0.47	0.46	0.77	0.53	0.38	0.35	0.43	0.44
24–48 h	0.24	0.28	0.48	0.62	0.22	0.24	0.39	0.37
48–72 h	0.10	0.11	0.22	0.22	0.13	0.09	0.19	0.18
72–96 h	0.07	0.07	0.12	0.15	0.06	0.05	0.13	0.12
96–120 h	0.04	0.05	0.08	0.11	0.04	0.03	0.09	0.11
120–144 h	0.03	0.04	0.05	0.08	0.03	0.04	0.07	0.07
144–168 h	0.03	0.02	0.08	0.06	0.02	0.02	0.05	0.05
Faeces:								
0–24 h	65.52	55.82	48.33	43.27	68.66	63.18	48.36	46.75
24–48 h	10.56	18.70	17.86	20.73	10.57	13.65	14.86	13.04
48–72 h	4.13	4.51	7.45	10.13	3.08	4.44	6.59	7.47
72–96 h	1.61	2.12	3.93	3.57	2.04	1.75	4.21	4.20
96–120 h	1.22	1.33	2.14	2.51	1.04	1.11	2.88	3.13
120–144 h	0.88	0.77	1.46	1.75	0.66	1.62	2.08	2.27
144–168 h	0.63	0.72	1.30	1.36	0.67	0.61	1.74	1.63
Total	90.39	90.75	93.00	93.61	92.90	91.51	92.26	94.93

From Rick et al. (2005b)

NA, not analysed; NQ, not quantifiable; radiolabel was not detected in tissues at a concentration exceeding the limit of quantitation (LOQ).

[a] Unlabelled compound as repeated oral doses at 10 mg/kg bw per day for 14 days, followed by a single radiolabelled dose.

to 226 mg/kg bw per day for 28 days, systemic bioavailability of factor L was 4–44% greater than that of factor J in all groups except females at 1200 ppm. In the latter, bioavailability of factor L was 16% lower, suggesting preferential absorption of factor L or faster elimination/first-pass metabolism of factor J. Although females were exposed to a relatively higher dietary concentration of spinetoram than males, owing to higher food consumption relative to body weight, serum concentrations of the two spinosyns in females were mostly lower than in males, suggesting lower systemic bioavailability, especially for factor L. Systemic bioavailability became nonlinear at the highest dose suggesting non-linear absorption (saturation), which was pronounced for factor L in females (Wilson et al., 2005a).

In a study of toxicity (also described in section 2.2), Fischer 344 rats were given diets containing spinetoram (purity, 95%; ratio, 64% factor J and 31% factor L)at doses ranging from 11 to 185 mg/kg bw per day for 28 days. Serum collected at termination indicated that recovery was greater than was proportional to the administered dose, this being more pronounced with factor J than with factor L.

This was an indication of saturation of elimination at high doses or more efficient first-pass elimination at lower doses, but absorption from the gastrointestinal tract appeared to be unaffected. A similar trend was observed in steady-state AUCs at 24 h, determined from three blood samples collected at 05:00, 10:00 and 17:00, determined 24 days after initiation of the feeding study. Plasma elimination half-lives increased with increasing dose; plasma elimination half-lives were 7 h and 9 h, 10 h and 12 h, and 32 h and 16 h for factor J and factor L at the lowest, intermediate and highest doses, respectively. The half-life of parent in this study was approximately 7 h at the lowest dose (Yano et al., 2004).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity with spinetoram are summarized in Table 3.

Spinetoram was of low acute oral and dermal toxicity in rats, giving oral and dermal median lethal dose (LD_{50}) values of > 5000 mg/kg bw.

The median lethal concentration (LC_{50}) for spinetoram administered by inhalation was > 5.44 mg/l, the highest aerosol concentration tested.

After oral dosing, clinical signs were observed only at doses of 2500 mg/kg bw and greater. Clinical signs after dosing orally and by inhalation included watery faeces, perineal soiling, perioral soiling and/or soiling of the coat (Carney et al., 2005a; Durando, 2007a).

(b) Dermal and ocular irritation and sensitization

The results of studies of irritation and sensitization with spinetoram are given in Table 4.

Studies of dermal and ocular irritation in New Zealand White (NZW) rabbits showed no irritation after dermal application and only transient ocular irritation immediately after instillation, which cleared within 24 h (Brooks & Golden, 2005a, 2005b; Durando, 2007c, 2007d).

In a local lymph node assay (LLNA) with BALB/c mice, spinetoram was shown to be a moderate skin sensitizer, while in a second LLNA with CBA/J mice (the recommended species for this assay, according to OPPTS 870.2600, OECD TG 429, and EC B.42 guidelines), spinetoram did not

Table 3. Acute toxicity of spinetoram

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Reference
Rat	F344/DuCrl	Females	Oral (gavage)	85.8% (64.6% factor J and 21.2% factor L)	> 5000	—	Carney et al. (2005a)
Rat	Fischer 344	Females	Oral (gavage)	86.3% (73.0% factor J and 13.3% factor L)	> 5000	—	Durando (2007a)
Rat	F344/DuCrl	Males and females	Dermal/topical	85.8% (64.6% factor J and 21.2% factor L)	> 5000	—	Carney et al. (2005b)
Rat	Fischer 344	Males and females	Dermal/topical	86.3% (sum of factor J and factor L)	> 5000	—	Durando (2007b)
Rat	F344/DuCrl	Males and females	Inhalation (nose only; 4 h)	85.8% (64.6% factor J and 21.2% factor L)	—	> 5.50	Hotchkiss et al. (2005)
Rat	Fischer 344	Males and females	Inhalation (nose only; 4 h)	84.5% (71.7% factor J and 12.9% factor L)	—	> 5.44	Krieger et al. (2007)

Table 4. Results of studies of sensitization and irritation with spinetoram

Species	Strain	Sex	Route	Purity (%)	Result	Reference
Rabbit	NZW	M & F	Dermal/topical	85.8% (64.6% factor J and 21.2% factor L)	No irritation	Brooks & Golden (2005a)
Rabbit	NZW	M & F	Dermal/topical	86.3% (73.0% factor J and 13.3% factor L)	Slight irritation	Durando (2007c)
Rabbit	NZW	M & F	Ocular (instillation)	85.8% (64.6% factor J and 21.2% factor L)	Transient irritation	Brooks & Golden (2005b)
Rabbit	NZW	M & F	Ocular (instillation)	86.3% (sum of factor J and factor L)	Transient irritation	Durando (2007d)
Mouse	BALB/c	F	Dermal/topical (local lymph node assay)	85.8% (64.6% factor J and 21.2% factor L)	Moderate sensitization	Woolhiser & Wiescinski (2006)
Mouse	CBA/J	F	Dermal/topical (local lymph node assay)	86.3% as the sum of (73.0% factor J and 13.3% factor L)	No sensitization	Wiescinski & Sosinski (2007)

F, female; M, male; NZW, New Zealand White

elicit a stimulation index that met the 3× threshold (i.e. a response that was three times greater than than elicited by the vehicle control), thus indicating a lack of dermal sensitization potential in mice in this assay (Woolhiser & Wiescinski, 2006; Wiescinski & Sosinski, 2007).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a short-term study of systemic toxicity, groups of five male and five female CD-1 mice were given diets containing spinetoram (purity, 95% active ingredient; 64% factor J and 31% factor L) at a concentration of 0, 50, 150, 450 or 1200 ppm (equal to 0, 8.28, 24.5, 75.1, and 183 mg/kg bw per day for males; and 0, 10.6, 31.3, 96.3, and 226 mg/kg bw per day for females) for 28 days. Steady-state serum concentrations of factor J and factor L were determined after 28 days for three males and three females per group. The homogeneity or stability of the diet was not determined.

Body weight was measured before exposure, twice during the first week and weekly during the remainder of the study. Food consumption and intake of spinetoram was determined twice during the first week and at least weekly thereafter for all mice. Ophthalmoscopic examinations were conducted before exposure and during necropsy. Haematology and clinical chemistry investigations were carried out at termination. Measurement of organ weights and examination for gross pathology were performed on all mice at termination. Histopathology examinations were carried out on mice in the control group and in the group at the highest dose at termination.

Terminal body-weight decreases of 10.4% for males and 3% for females, relative to controls, were statistically significant in mice at 1200 ppm, the highest dose. The body-weight gain of mice at 1200ppm was only 50% (males) and 83% (females) that of mice in the control groups. Treatment-related decreases in food consumption occurred in males and females at 1200 ppm.

The primary treatment-related effect was cytoplasmic vacuolation of the parenchymal cells, epithelial cells, macrophages (with increased numbers), and fibroblasts of various organs in mice at 1200 ppm, with more subtle effects of a similar nature in mice at 450 ppm. Other treatment-related effects included hyperplasia of the glandular mucosa of the stomach in females at 1200 ppm and in males at a dietary concentration of 450 ppm or greater, degeneration with regeneration of skeletal

muscle fibers in mice at 450 ppm or greater, and very slight hypertrophy of the zona fasciculata in adrenal glands of males at 1200 ppm.

Administration of spinetoram at a dietary concentration of 1200 ppm caused increases in absolute and relative liver and spleen weights. Treatment-related increases in absolute and relative adrenal weights (relative adrenal weights increased by 75%) occurred in males at 450 ppm or greater.

Although there was no decrease in the erythrocyte count, mice receiving spinetoram at 1200 ppm had slight microcytic anaemia, as shown by decreases in eythrocyte cell volume, haemoglobin (10%), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV). Similar but more subtle changes in erythrocyte cell volume and haemoglobin (11%) occurred in male mice at 450 ppm. All males and one of five females at 1200 ppm had splenic extramedullary haematopoiesis. Mice at 450 ppm or greater had an increase in the percentage of monocytes, which correlated with the macrophage response that occurred in many tissues. Males at 450 ppm or greater also had an increase in neutrophil numbers consistent with the inflammatory response that occurred. The results of haematology analyses are shown in Table 5.

At a dietary concentration of 1200 ppm, spinetoram caused significant elevation of hepatobiliary enzyme activity in serum (alkaline phosphatase: males, +37%; and aspartate aminotransferase (AST): males, +195%; females, +148%). Male and female mice at 1200 ppm had a slight statistically significant (for males and females combined) treatment-related decrease in albumin concentration that was interpreted to be the result of treatment-related decrements in food consumption at this dietary concentration.

Table 5. Selected haematology findings in mice given diets containing spinetoram for 28 days

Dietary concentration (ppm)	Parameter					
	Erythrocyte count (R6/µl)	Haemoglobin (g/dl)	Haematocrit[c] (%)	Mean corpuscular volume (fl)	Mean corpuscular haemoglobin (pg)	Mean corpuscular haemoglobin concentration (g/dl)
Males						
0	9.57	15.8	49.9	52.2	16.5	31.6
50	9.54	15.3	47.9	50.2	16.1	32.0
150	9.39	15.3	48.3	51.5	16.2	31.6
450	8.62*	14.1*	45.0*	52.1	16.4	31.4
1200	9.55	14.2	45.7*	47.9	14.9	31.1
Range for historical controls[a]	8.73–9.48	14.5–15.9	47.0–49.0	52.4–54.3	15.8–17.0	29.8–32.0
Females						
0	9.28	15.7	48.9	52.7	16.9	32.1
50	9.65	16.0	50.3	52.3	16.7	31.8
150	9.67	15.6	50.2	51.9	16.2	31.2
450	9.30*	15.1*	48.0	51.6	16.3	31.5
1200	9.40*	14.1*	45.7*	48.8	15.1	30.9
Range for historical controls[a]	0.00–9.54[b]	15.1–16.0[b]	45.5–51.4	51.3–53.0	15.0–17.1	29.0–33.4

From Wilson et al. (2005a)

[a] Values for historical control groups from the same laboratory for six studies of dietary toxicity in CD-1 mice during the past 5 years.

[b] Values for historical control groups from the same laboratory for five studies of dietary toxicity in CD-1 mice during the past 5 years.

[c] Erythrocyte volume fraction = haematocrit/100

*Dunnett test statistically identified at alpha = 0.05.

The no-observed-adverse-effect level (NOAEL) for CD-1 mice given diets containing spinetoram for 28 days was 150 ppm , equal to 24.5 mg/kg bw per day and 31.3 mg/kg bw per day for males and females, respectively, on the basis of haematological changes in males and cytoplasmic vacuolation of the parenchymal cells, epithelial cells, macrophages (with increased numbers), and fibroblasts of various organs in mice at 450 ppm (Wilson et al., 2005a).

In a short-term study of toxicity, groups of ten male and ten female CD-1 mice were given diets containing spinetoram (purity, 83.0%; 62% factor J and 21.0% factor L) at a concentration of 0, 50, 150 or 450 ppm (equal to 0, 7.5, 22.8, and 70.5 mg/kg bw per day for males and 0, 10.2, 29.6, and 89.9 mg/kg bw per day for females) for at least 90 days. Parameters evaluated were daily observations, detailed clinical observations, ophthalmological examinations, body weight, food consumption, haematology, clinical chemistry, weights of selected organs, and gross and histopathology examinations. Diet homogeneity and stability were not determined.

Males at 450 ppm had treatment-related decrements in food consumption of up to 11% and decreases in body weights of up to 8%, relative to controls. The body-weight gains of males at this dose were substantially lower than those of the controls throughout the study, with a 24% reduction by study termination.

Administration of spinetoram at dietary concentration of 450 ppm caused slight treatment-related microcytic hypochromic anaemia, as shown by a decrease (approximately 5%) in the erythrocyte count, which did not reach statistical significance, and by statistically significant decreases in haemoglobin concentration (≤ 10%) and erythrocyte volume fraction, decreased mean corpuscular volume, and increased reticulocyte counts (statistically significant in males only). Leukocyte count was statistically significantly elevated in females at 150 and 450 ppm. Compared with controls, males and females at 450 ppm had treatment-related increases in the activity of serum AST and females at 450 ppm showed increases in the activity of alanine aminotransferase (ALT).

Males and females at 450 ppm had treatment-related increases in mean absolute (31–38%) and relative spleen weights (40%) that were statistically significant. The increase in spleen weight was attributed to splenic extramedullary haematopoiesis. Males and females at 450 ppm had treatment-related increases in mean absolute (11–17%) and relative (18–19%) liver weights. Only the change in relative liver weight was statistically significant.

The primary treatment-related histological change was cytoplasmic vacuolation of parenchymal cells, epithelial cells, macrophages and fibroblasts in numerous organs in mice at 450 ppm. Other treatment-related histological changes included hyperplasia of the glandular mucosa of the stomach, multifocal degeneration and regeneration of skeletal muscle fibers and renal tubular epithelium, and a slight increase in splenic extramedullary haematopoiesis in males and females at 450 ppm. The only treatment-related histological change in mice at 150 ppm was subtle vacuolation of the tubules of the caput epididymis in males, slight splenic extramedullary haematopoiesis and very slight dilatation of the glandular stomach in females. Histopathology findings are summarized in Table 6.

The NOAEL was 50 ppm, equivalent to 7.5 mg/kg bw per day for males and 10.2 mg/kg bw per day for females, on the basis of slight splenic extramedullary haematopoiesis in females and slight vacuolation of the tubules of the caput epididymis of males at 150 ppm (Wilson et al., 2005b).

Rats

In a 28-day study of toxicity, groups of five male and five female Fischer 344 rats were given diets containing spinetoram (purity, 95% active ingredient; 64% factor J and 31% factor L) at a concentration of 0, 120, 500, 1500 ppm (females only) or 2000 ppm (males only) for at least 28 days. These concentrations corresponded to intakes of 0, 11.4, 48.4 and 185 mg/kg bw per day for males and 0, 11.7, 48.2 and 142 mg/kg bw per day for females. Body weight, food consumption, compound

Table 6. Selected histopathology findings in mice given diets containing spinetoram for up to 90 days

Finding	Dietary concentration (ppm)							
	Males (n = 10 per dose)				Females (n = 10 per dose)			
	0	50	150	450	0	50	150	450
Epididymides								
Vacuolization, epithelium, multifocal:								
Very slight	0	0	6	0	—	—	—	—
Slight	0	0	0	10	—	—	—	—
Kidneys								
Degeneration with regeneration, tubule, multifocal: slight	0	0	0	6	0	0	0	3
Liver								
Vacuolization, macrophages, perivascular, multifocal: very slight	0	0	0	0	0	0	0	6
Lymph node, mediastinal								
Vacuolization, macrophages: very slight	0	0	0	10	0	0	0	8
Lymph node, mesenteric								
Vacuolization, macrophages: very slight	0	0	0	10	0	0	0	9
Sinus histiocytosis, increased: slight	0	0	0	2	0	0	0	3
Skeletal muscle								
Degeneration with regeneration, muscle fiber, multifocal:								
Very slight	0	0	0	10	0	0	0	0
Slight	0	0	0	0	0	0	0	9
Spleen								
Extramedullary haematopoeisis, increased: slight	0	0	0	7	1	1	3	7
Vacuolization, macrophages: very slight	0	0	0	7	1	0	0	6
Stomach								
Dilatation, glandular, glandular mucosa, multifocal: very slight	1	0	0	8	0	0	2	7
Hyperplasia, glandular mucosa, diffuse:								
Very slight	0	0	0	3	0	0	0	2
Slight	0	0	0	7	0	0	0	7
Inflammation, chronic active, glandular mucosa, multifocal:								
Very slight	1	0	0	4	0	0	0	4
Slight	0	0	0	0	0	0	0	2

From Wilson et al. (2005b)

intake, ophthalmoscopic examination, haematology, clinical chemistry, urine analysis, necropsy and histopathology were evaluated.

Analyses to confirm all doses administered were determined before exposure. The homogeneity of the diet containing spinetoram at the lowest and highest concentrations was determined concurrently. A study of toxicity previously conducted with a structurally similar compound had shown that test material was stable in the diet for up to 40 days. The stability of spinetoram was assumed to be similar, and was therefore not investigated further. Samples of all prepared diets were retained and stored frozen at approximately –20 °C for possible future analyses.

There were no treatment-related effects in clinical observations, ophthalmic observations, haematology, coagulation, clinical chemistry, or urine-analysis parameters.

Males at the highest dose had decreases in body weight relative to controls (4.6%), and body-weight gain (8.6%) at the end of the study that were consistent with the slightly lower food consumption in this group. Males at 2000 ppm had higher relative weights of heart, spleen (both statistically significant) and kidney. Females at 1500 ppm had higher absolute and relative heart, spleen, liver and kidney weights that were statistically significantly different from values for the controls. Spleen weights (19% greater than values for controls) were interpreted to be treatment-related as they were outside the range of values for historical controls and because treatment-related histopathological effects were also observed in the spleen.

Treatment-related histopathological effects occurred in the thyroid and kidneys of males and females in the groups receiving the highest dose and consisted of a slight vacuolation of the follicular epithelial cells of the thyroid and a very slight vacuolation of the renal tubular epithelial cells. Two females at 500 ppm also had a very slight vacuolation of the renal tubular epithelium. Males and females at the highest dose had slight accumulation of macrophages/histiocytes within the white pulp of the spleen and females had an increase in spleen weight. Slight splenic histiocytosis also occurred in one female at 500 ppm. In addition, males and females at the highest dose had very slight or slight accumulation of macrophages/histiocytes in the cortex of mesenteric lymph nodes. Histopathology findings are summarized in Table 7.

The NOAEL was 500 ppm, equal to 48 mg/kg bw per day, on the basis of minor histopathological changes in several organs observed in rats at the highest dietary concentration. The low incidence, and minimal severity of the vacuolation in the kidney and histiocytosis in the spleen observed in a few females at 48 mg/kg bw per day were not considered to be adverse effects (Yano et al., 2004).

Table 7. Selected histopathology findings in rats given diets containing spinetoram for 28 days

Finding	Dietary concentration (ppm)							
	Males (n = 5 per group)				Females (n = 5 per group)			
	0	120	500	2000	0	120	500	1500
Thyroid								
Vacuolation, follicular cells, cytoplasmic:								
Very slight	1	0	1	0	0	0	1	0
Slight	0	0	0	5	0	0	0	5
Spleen								
Aggregates of macrophages-histiocytes, periarteriolar lymphoid sheath:								
Focal, very slight	1	1	1	0	0	0	0	0
Multifocal, very slight	0	0	0	4	0	0	1	4
Lymph node, mesenteric								
Aggregates of macrophages-histiocytes, cortex:								
Focal, very slight	1	0	0	4	0	0	0	5
Multifocal, slight	0	0	0	1	0	0	0	0
Kidney								
Vacuolation increased, tubular epithelium: very slight	0	0	0	5	0	0	2	5

From Yano et al. (2004)

In a 90-day study to evaluate potential systemic toxicity, groups of 10 male and 10 female Fischer 344 rats were given diets formulated to supply spinetoram (purity, 83.0%; 62.0% factor J and 21.0% factor L) at a concentration of 0, 120, 500, 1000, 2000 ppm, or 4000 ppm (females only) for at least 90 days. These dietary concentrations were equal to doses of 0, 8.49, 34.7, 70.6, and 137 mg/kg bw per day for males and 0, 10.1, 42.4, 85.0, 170, and 332 mg/kg bw per day for females. Additional groups of 10 males and 10 females were given spinetoram at 0 or 1000 ppm for 90 days, and were then maintained on control diet for an additional 4 weeks to assess the potential reversibility of treatment-related effects. Parameters evaluated included cage-side observations, detailed clinical observations, ophthalmological exams, body weight, food consumption, haematology, clinical chemistry (including thyroid-hormone analysis), urine analysis, selected organ weights, gross and histopathological examinations, and electron microscopy.

The homogeneity of the diet containing spinetoram at the lowest and highest doses for females was determined before exposure, near the middle, and at the end of the study. Stability was established for 62 days in diet containing spinetoram at concentrations ranging from 0.0005% to 4%. The concentrations of spinetoram were confirmed by LC/MS/MS analysis in samples of diet containing spinetoram at all doses (plus control diet and pre-mix) analysed before exposure, near the middle, and at the end of the study.

Males at 2000 ppm showed a decrease in body-weight gain of 10.2% at the end of the dosing phase, while females at 2000 or 4000 ppm had body-weight gain decreases of 7.9% and 14.8%, respectively. Food consumption of males at 2000 ppm and females at 4000 ppm was also less than that of the controls.

Females at 2000 or 4000 ppm showed decreases in erythrocyte parameters (haemoglobin concentration: 2000 ppm, –8%; 4000 ppm, –11%, erythrocyte volume fraction, MCV and MCHC) and higher reticulocyte counts (2000 ppm, +29%; 4000 ppm, +41%). Changes in these parameters were also observed in females at 1000 ppm, reaching statistical significance for haemoglobin and erythrocyte volume fraction. At this dose, the reticulocyte count was 17% higher than that in the controls. There were no decreases in erythrocyte parameters in the males. The leukocyte counts of females at 1000 ppm or greater were also higher than those of the controls (+30%, +53% and +80%, respectively, statistically significant at the two higher doses). Males at 2000 ppm and females at 2000 ppm or greater had higher activities of serum liver enzymes (ALT in males; AST in males and females). The triglyceride concentrations of females at 500 ppm or greater were statistically significantly lower than those of the controls and showed a dose-dependent relationship, while cholesterol concentrations were reduced at 2000 ppm or greater. Females at 4000 ppm also had a slightly higher alkaline phosphatase activity relative to that of controls.

Concentrations of triiodothyronine (T3) were slightly, statistically significantly, reduced in females at 2000 and 4000 ppm. Concentrations of thyroxin (T4) were slightly, statistically significantly, reduced in females at dietary concentrations of 500 ppm, 1000 ppm and 2000 ppm, but not at the highest concentration, 4000 ppm, where concentrations were not dissimilar to values for the controls. There were no changes in concentrations of thyroid-stimulating hormone (TSH).

Microscopic treatment-related effects consisting of the presence of aggregates of macrophages/histiocytes occurred in numerous lymphoid tissues, including the spleen, lymph nodes, Peyer patches of the jejunum or ileum, and thymus, and the liver and bone marrow. Vacuolation of parenchymal cells occurred in the thyroid gland and kidney, and there was skeletal muscle degeneration involving multiple muscles. Females were more affected than males, with effects on the thyroid, spleen, bone marrow, jejunum, liver, kidney and mesenteric and mediastinal lymph nodes at the lowest observed-effect level (LOEL). Histopathology findings are summarized in Tables 8 and 9.

Evaluation of the kidneys by electron microscopy indicated that females at 4000 ppm had vacuoles within tubular epithelial cells that contained a flocculent material or membranous whorls.

Table 8. Selected histopathology findings in female rats given diets containing spinetoram for 90 days

Finding	Dietary concentration (ppm)					
	0	120	500	1000	2000	4000
Lymph node, mediastinal (No. examined)	10	10	9	9	10	10
Aggregates of macrophages-histiocytes, multifocal:						
Very slight	2	0	1	5	8	8
Slight	0	0	0	0	2	1
Lymph node, mesenteric (No. examined)	10	10	10	10	10	10
Aggregates of macrophages-histiocytes						
Focal, very slight	2	3	1	0	0	0
Multifocal, moderate	0	0	0	4	8	10
Spleen (No. examined)	10	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:						
Slight	0	0	0	2	5	4
Moderate	0	0	0	0	3	3
Bone marrow (No. examined)	10	10	10	10	10	10
Aggregates of macrophages-histiocytes:						
Hindlimb, multifocal, very slight	0	0	3	6	5	4
Hindlimb, multifocal, slight	0	0	0	1	4	6
Sternum, multifocal, very slight	0	0	2	4	9	8
Vertebrae, focal, very slight	0	0	2	3	3	1
Vertebrae, multifocal, very slight	0	0	1	1	3	6
Kidney (No. examined)	10	10	10	10	10	10
Vacuolization, tubules, slight	0	0	0	0	6	8
Thyroid (No. examined)	10	10	10	10	10	10
Vacuolization, follicle, epithelial cell, slight	0	0	0	7	10	10
Depletion, with altered tinctorial properties, colloid, very slight	0	0	0	4	4	3

From Yano et al. (2005)

These effects were consistent with those observed in animals given agents known to be cationic amphiphilic drugs and establish spinetoram as a cationic amphiphilic compound.

The potential to recover from the effects induced by spinetoram was investigated in male and female rats given spinetoram at a dietary concentration of 1000 ppm for 90 days, followed by control food for 28 days. Variable degrees of recovery occurred during the recovery phase. Complete recovery was noted for a number of effects including changes in erythrocyte parameters, lower concentrations of triglycerides, higher relative liver weights, higher relative heart weight, and microscopic effects involving the ileum (males), jejunum (males), kidney (females), liver (males), spleen (males), skeletal muscle and thymus. Partial recovery occurred for relative spleen weights and microscopic effects involving the kidneys (males), spleen (females), jejunum (females), liver (females) and thyroid glands. In the recovery phase, histopathological findings related to mesenteric lymph nodes were almost un-changed from those in rats not allowed a recovery phase while microscopic effects in ileum in females were more severe than those in rats without a recovery phase. ALT activity in males at 1000 ppm remained statistically significantly elevated at the end of the recovery phase, at levels similar to those found at the end of the dosing period.

Table 9. Selected histopathology findings in male rats given diets containing spinetoram for 90 days

Finding	Dietary concentration (ppm)				
	0	120	500	1000	2000
Lymph node, mesenteric (No. examined)	9	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Very slight	3	2	5	6	0
Slight	0	0	0	2	8
Liver (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Very slight	0	0	0	0	4
Slight	0	0	0	0	3
Thymus (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes:					
Focal, very slight	3	0	1	3	3
Multifocal, very slight	0	0	0	2	5
Kidney (No. examined)	10	0	10	10	10
Hyaline droplet formation, decreased, proximal convoluted tubule:					
Slight	2	—	1	4	4
Moderate	0	—	0	1	6
Thyroid (No. examined)	10	10	10	10	10
Vacuolization, follicle, epithelial cell, slight	0	0	0	1	6
Depletion, with altered tinctorial properties, colloid, very slight	0	0	0	1	5

From Yano et al. (2005)

The NOAEL was 500 ppm for males and 120 ppm for females (equivalent to 34.7 mg/kg bw per day for males and 10.1 mg/kg bw per day for females) on the basis of changes in haematological parameters (reticulocyte and leukocyte counts) and histopathological findings in the bone marrow (Yano et al., 2005).

In a short-term dietary study of toxicity, groups of eight male and eight female CD (Sprague-Dawley) rats were fed diets providing spinetoram (purity, 83.0% active ingredient; 62.0% factor J and 21.0% factor L) at a dose of 0, 10, 50, 100, or 150 mg/kg bw per day for 90 days. This study was designed as a pilot study for dose selection for the two-generation study of reproductive toxicity (Carney et al., 2006) and was not designed to satisfy all regulatory requirements for a short-term dietary study of oral toxicity in rats. Body weight, food consumption, compound intake, clinical chemistry, urine analysis, necropsy and histopathology were evaluated.

The homogeneity of the diets determined during the third week of study. Stability was established for 62 days in diet containing spinetoram at concentrations ranging from 0.0005% to 4%.

There were no treatment-related clinical signs of toxicity at any dose. Treatment-related increases in organ weights were observed in males at 150 mg/kg bw per day and females at 100 or 150 mg/kg bw per day. In males, there were statistically significant increases in relative weights of the heart and spleen. Absolute spleen weight was increased non-significantly in males and females. In females at 150 mg/kg bw per day, relative weights of the heart and kidney, and absolute and relative weights of the liver and spleen were increased. The changes in spleen weight did not reach statistical significance. At 100 mg/kg bw per day, relative weights of the heart and absolute and relative

weights of the liver were increased, although the change in absolute liver weight was not statistically significant.

Treatment-related histological alterations were limited to the thyroid, spleen, and kidneys. Thyroid follicular epithelial cells in most males and females at 150 mg/kg bw per day were enlarged and distended with fine cytoplasmic vacuoles. This histopathological effect was also present with moderate severity in two males and three females at 100 mg/kg bw per day. Treatment-related, diffuse vacuolation of a lesser degree (slight) was present in the thyroid of males and females at 50 or 100 mg/kg bw per day. Thyroid-hormone analysis indicated differences from control values in concentrations of TSH (increased), T4 and T3 (decreased) for males at 10, 50 or 100 mg/kg bw per day. However, the biological significance of the decrease in T_4 was equivocal as there was no dose–response pattern. In females, there were no changes in TSH concentrations, but there was a statistically significant decrease in T4 concentrations in rats receiving spinetoram at 100 mg/kg bw per day or greater.

The NOAEL was 10 mg/kg bw per day on the basis of slight vacuolation in the thyroid of males and females receiving spinetoram at 50 mg/kg bw per day (Wilson et al., 2005a).

In a short-term study of potential systemic toxicity, groups of 10 male and 10 female Fischer 344 rats were given diets containing spinetoram (purity, 86.3%; 73.0% factor J and 13.3% factor L; 85 : 15 ratio of J:L) at a concentration of 0, 120, 500, 1000 or 2000 ppm for 90 days. These dietary concentrations corresponded to doses of 0, 8, 35, 69 or 137 mg/kg bw per day for males and 0, 9, 35, 71, or 142 mg/kg bw per day for females. Body weight, food consumption, compound intake, ophthalmoscopic examination, haematology, clinical chemistry, urine anaalysis, necropsy and histopathology were evaluated. A previously conducted study with spinetoram (75 : 25 ratio) indicated that the test material was stable in the diet for at least 62 days at concentrations ranging from 0.0005% to 4%. The doses received and homogeneity of the diets administered were confirmed analytically.

There were slight reductions in mean body weights and body-weight gains in males at 2000 ppm (from day 50), but these differences were not statistically significant. Food consumption was not consistently affected by treatment.

Males at 2000 ppm had treatment-related changes in various erythrocyte parameters: mean leukocyte count and mean reticulocyte counts increased by 21%, and 14%, respectively. Females at 1000 or 2000 ppm had treatment-related decreases in mean platelet counts. Treatment-related alterations in leukocyte parameters for females consisted of a higher mean total leukocyte count at 2000 ppm (+26%), a lower percentage of neutrophils at 1000 or 2000 ppm, and a higher percentage of basophils and large unstained cells and increases in mean reticulocyte counts (+52%) at 2000 ppm.

Males at 1000 or 2000 ppm had treatment-related increases in mean serum alanine and AST activities. Treatment-related increases in alkaline phosphatase activity were seen in males at 2000 ppm. Females at 2000 ppm had a treatment-related increase in mean AST activity. Alkaline phosphatase activity was also slightly elevated in this group.

Microscopic treatment-related effects consisting of the presence of aggregates of macrophages-histiocytes occurred in lymphoid tissues, including the spleen, lymph nodes, Peyer patches of the jejunum or ileum, and thymus, and the liver and bone marrow. Vacuolization of parenchymal cells occurred in the thyroid gland and kidney, and muscle degeneration was noted in the heart and skeletal muscle of the larynx. Females were more affected than males, with thyroid, bone marrow, liver, thymus, and mesenteric and mediastinal lymph node effects at the lowest-observed-effect level (LOEL) of 500 ppm. Histopathology findings are summarized in Tables 10 and 11.

The NOAEL for spinetoram (85:15 ratio) was 500 ppm in males and 120 ppm in females (equivalent to 35 mg/kg bw per day for males and 9 mg/kg bw per day for females) on the basis of histopathological findings in lymph nodes and bone marrow at higher doses (Stebbins & Card, 2007).

Table 10. Selected histopathology findings in male rats given diets containing spinetoram for 90 days

Finding	Dietary concentration (ppm)				
	0	120	500	1000	2000
Lymph node, mediastinal (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Very slight	3	1	1	6	8
Slight	0	0	0	0	2
Lymph node, mesenteric (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal, slight	0	0	0	5	10
Spleen (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Slight	0	0	0	0	2
Moderate	0	0	0	0	6
Thymus (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal, very slight	0	0	0	1	8
Bone marrow (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes:					
Hindlimb, multifocal, very slight	0	0	0	0	7
Hindlimb, multifocal, moderate	0	0	0	0	1
Sternum, multifocal, very slight	0	0	0	0	2

From Stebbins & Card (2007)

Dogs

Groups of two male and two female beagle dogs were fed diets containing spinetoram (purity, 83.0% active ingredient; 62.0% factor J and 21.0% factor L) at a concentration of 0, 200, 900, or 2000 ppm (equal to 0, 5.9, 30.9, and 65.0 mg/kg bw per day in males, and 0, 8.1, 35.1, and 62.3 mg/kg bw per day in females) for 28 days. Parameters evaluated included daily observations, detailed clinical observations, ophthalmic examinations, body weight, food consumption, prothrombin time, clinical chemistry, haematology, urine analysis, selected organ weights, and gross and histopathological examinations. The homogeneity and stability of the diet and doses administered were confirmed analytically.

There were no treatment-related effects on daily observations, detailed clinical observations, ophthalmic examinations, prothrombin time, urine analysis, or gross pathology examinations.

Treatment-related changes in numerous parameters were noted at 900 and 2000 ppm. Dogs at these doses had lower body weight (6% for females and 8% for males) and body-weight gain, relative to controls. One female at 900 ppm and two females at 2000 ppm lost body weight over the duration of the study (starting from day 4) and food consumption in these dogs was reduced. Food consumption in males was unaffected.

Alterations in erythrocyte, leukocyte, and platelet parameters reflective of a non-regenerative anaemia (erythrocytes, –15%; haemoglobin, –22%; erythrocyte volume fraction, –17%; leukocytes, –52%; and platelets, –73%) at 2000 ppm in males. Similar changes were observed at 2000 ppm in females, and slightly less marked changes were observed at 900 ppm in females. Increases in serum ALT (+41%) and asparate aminotransferase (+61%) activities occurred in males at 2000 ppm, with similar changes in asparate aminotransferase in females at 900 or 2000 ppm.

Table 11. Selected histopathology findings in female rats given diets containing spinetoram for 90 days

Finding	Dietary concentration (ppm)				
	0	120	500	1000	2000
Lymph node, mediastinal (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Very slight	1	1	7	7	6
Slight	0	0	0	1	4
Lymph node, mesenteric (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Slight	2	0	6	10	8
Moderate	0	0	0	0	2
Spleen (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal					
Slight	0	0	0	3	7
Moderate	0	0	0	0	2
Thymus (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal, very slight	0	0	1	5	8
Bone marrow (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes:					
Hindlimb, multifocal, slight	0	0	0	1	8
Sternum, multifocal, very slight	0	0	2	4	9
Vertebra, multifocal, very slight	0	0	0	1	2

From Stebbins & Card (2007)

Treatment-related increases in absolute and/or relative liver weights were seen at 900 and 2000 ppm (relative liver weight, +45% in males at 2000 ppm). One male and one female at 900 ppm, and one female at 2000 ppm had treatment-related decreases in absolute and relative weight of the thymus. The lower weights of the thymus corresponded to atrophy of the thymic cortex observed microscopically in the two affected females.

Histologically, treatment-related vacuolization (very slight or slight severity) of macrophages within lymphoid tissue occurred in the caecum, gallbladder, ileum, larynx, lymph nodes, nasal tissue, rectum, spleen, stomach, thymus, and tonsils of males and females at 900 or 2000 ppm. In addition, very slight to slight necrosis and moderate diffuse hyperplasia of mononuclear cells were noted in the bone marrow at 900 and 2000 ppm. Extramedullary haematopoiesis of the spleen, noted in one female at 900 ppm and one male and one female at 2000 ppm, was interpreted to be a response to the bone-marrow necrosis and anaemia at these doses. Hyperplasia and hypertrophy (very slight or slight severity) of Kupffer cells in the liver occurred in all dogs at 900 or 2000 ppm. Some of the Kupffer cells of dogs at 2000 ppm had treatment-related cytoplasmic vacuolization. All dogs at 2000 ppm had treatment-related aggregates of alveolar macrophages in the lungs. Histopathology findings are summarized in Table 12.

The NOAEL for systemic toxicity in beagle dogs given diets containing spinetoram for 28 days was 200 ppm, which corresponded to 5.9 mg/kg bw per day for males and 8.1 mg/kg bw per day for females, on the basis of haematological, biochemistry and histopathological findings at higher doses (Stebbins & Brooks, 2004).

Table 12. Selected histopathology findings in dogs given diets containing spinetoram for 28 days

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	200	900	2000	0	200	900	2000
Bone marrow								
Hyperplasia, mononuclear cell, diffuse, moderate	0	0	0	2	0	0	1	2
Necrosis, multifocal:								
Very slight	0	0	1	1	0	0	0	0
Slight	0	0	0	1	0	0	2	2
Vacuolization, macrophages:								
Very slight	0	0	1	0	0	0	0	0
Slight	0	0	0	2	0	0	2	2
Liver								
Hyperplasia and hypertrophy, Kupffer cell:								
Very slight	0	0	2	0	0	0	2	0
Slight	0	0	0	2	0	0	0	2
Vacuolization, Kupffer cell, very slight	0	0	0	2	0	0	0	2
Spleen								
Extramedullary haematopoiesis:								
Very slight	0	0	0	0	0	0	0	1
Slight	0	0	0	1	0	0	1	1
Vacuolization, macrophages, white pulp: very slight	0	0	0	2	0	0	0	2

From Stebbins & Brooks (2004)

Groups of four male and four female beagle dogs were fed diets containing spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) at a concentration of 0, 150, 300, or 900 ppm (equal to 0, 5.73, 9.82, and 27.1 mg/kg bw per day in males, and 0, 4.97, 10.2, and 31.0 mg/kg bw per day in females) for 90 days. The homogeneity and stability of the diet and actual doses administered were confirmed analytically.

Males at 300 or 900 ppm, and females at 900 ppm had treatment-related lower mean body weights compared with controls (males, –14% and –17%, respectively) and body-weight gains during most of the study (males, –87%; females, –24%). There was appreciable inter-individual variation in the magnitude of these changes, particularly in males, and they did not achieve statistical significance. Food consumption was not affected by treatment.

Decreases in mean leukocyte count, erythrocyte count (males, –18%; females, –7%; at 90 days), haemoglobin concentration (males, –20%; females, –15%; at 90 days), erythrocyte volume fraction, and platelet count were reported in males and females at 900 ppm associated with a non significant increase of reticulocytes (males, 36%; females, 105%; at 90 days). Only the change in haemoglobin concentration was statistically significant.

Clinical-chemistry alterations consisted of a statistically non-significant increase in alkaline phosphatase activity in males given 300 or 900 ppm. AST activity was slightly, although statistically significantly, increased in males and females at 900 ppm.

Treatment-related increases in absolute and relative weights of the liver were noted in males (+56%) and females (+23%) at 900 ppm. In males and females, absolute and relative weights of the thymus were statistically significantly reduced at 900 ppm, and absolute weights were statisti-

cally significantly reduced at 300 ppm, to below the limit of the range for historical controls for the laboratory.

Histologically, treatment-related vacuolization (very slight or slight severity) of macrophages within lymphoid tissue occurred in the caecum, colon, duodenum, ileum, jejunum, lungs, lymph nodes, nasal tissue, rectum, spleen, stomach, and tonsil of males and females at 300 or 900 ppm (Table 13). Very slight vacuolization of macrophages within lymphoid tissue also occurred in the ileum, jejunum, lymph nodes, nasal tissues, and rectum of some males at 150 ppm. Treatment-related arteritis or perivascular inflammation (very slight, slight, or moderate severity) occurred in numerous tissues of some males and females at 300 or 900 ppm. The more severe arteritis was frequently accompanied by necrosis of the arterial walls, with occasional associated haemorrhage. Very slight to moderate bone-marrow necrosis was present in some males and females at 300 or 900 ppm. Extramedullary haematopoiesis of the spleen and liver in some females at 300 or 900 ppm was interpreted to be a response to the bone-marrow necrosis and/or aanemia at these doses. Hyperplasia and hypertrophy (very slight or slight severity) of Kupffer cells, and vacuolization of Kupffer cells occurred in the liver of some males and females at 300 or 900 ppm.

Table 13. Incidence of vacuolation of macrophages in dogs given diets containing spinetoram for 90 days

Tissue or organ[a]	Severity	Dietary concentration (ppm)							
		Males (n = 4 per group)				Females (n = 4 per group)			
		0	150	300	900	0	150	300	900
Bone marrow	Very slight	0	0	0	0	0	0	0	1
	Slight	0	0	2	3	0	0	1	3
Caecum	Very slight	0	0	3	3	0	0	2	1
Colon	Very slight	0	0	2	1	0	0	2	1
Duodenum	Very slight	0	0	0	0	0	0	0	2
Ileum	Very slight	0	2	3	3	0	0	2	2
	Slight	0	0	0	1	0	0	1	2
Jejunum	Very slight	0	1	2	0	0	0	0	0
	Slight	0	0	0	1	0	0	0	1
Larynx	Very slight	0	0	1	0	0	0	0	1
Lungs	Very slight	0	0	0	3	0	0	0	1
Lymph node, mediastinal	Very slight	0	0	3	3	0	0	1	2
	Slight	0	0	0	1	0	0	0	0
Lymph node, mesenteric	Very slight	0	1	4	2	0	0	2	4
	Slight	0	0	0	2	0	0	0	0
Nasal tissue	Very slight	0	1	3	1	0	0	1	3
	Slight	0	0	0	2	0	0	0	1
Rectum	Very slight	0	2	3	4	0	0	1	3
Spleen	Very slight	0	0	1	4	0	0	2	0
Stomach	Very slight	0	0	1	2	0	0	0	1
Tonsil: vacuolation	Very slight	0	0	3	0	0	0	3	1
	Slight	0	0	0	4	0	0	0	3

From Stebbins & Brooks (2005)

[a] Vacuolated macrophages in the bone marrow were present adjacent to sites of necrosis. All other vacuolated macrophages were present in lymphoid tissue of the affected organs and tissues.

The NOAEL was 150 ppm, equivalent to 5.73 mg/kg bw per day in males, and 4.97 mg/kg bw per day in females, on the basis of histopathological findings (vacuolization, arteritis or perivascular inflammation) and extramedullary haematopoiesis at 300 ppm. The occurrence of very slight vacuolization of macrophages in lymphoid tissues of a few male dogs at 150 ppm was not considered to be adverse (Stebbins & Brooks, 2005).

Groups of four male and four female beagle dogs were fed diets containing spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) at a concentration of 0, 50, 100, or 200 ppm for 1 year. These concentrations were equal to doses of approximately 0, 1.6, 3.0, and 5.4 mg/kg bw per day in males, and 0, 1.3, 2.5, and 5.8 mg/kg bw per day in females. Parameters evaluated included daily cage-side observations, weekly detailed clinical observations, ophthalmology, body weights, food consumption, clinical chemistry, haematology, prothrombin time, urine analysis, selected organ weights, and gross and histopathology examinations. The homogeneity and stability of the diet and actual doses administered were confirmed analytically.

There were no treatment-related effects on daily observations, detailed clinical observations, ophthalmic examinations, body weights, food consumption, haematology, clinical pathology parameters, or gross pathology examinations. The mean absolute and relative weights of the liver of males at 200 ppm were 17.7% and 19.4% higher than those of the controls, respectively. These increases were not statistically significant, but were considered most likely to be treatment-related as the liver weights were above the range for historical controls for the laboratory. There were no associated clinical pathology or microscopic changes.

Arteritis in one male and one female at 200 ppm was the only potentially treatment-related histopathological effect noted (Table 14). Arteritis occurred bilaterally in the epididymides of one male at 200 ppm, and in the thymus, thyroid, larynx, and urinary bladder of one female at 200 ppm. Although no arteritis was observed in the controls in this study, the incidence in the treated groups was within the range for historical controls. The arteritis was accompanied by necrosis of the arterial walls in the affected dogs. No treatment-related vacuolation of macrophages was observed in this study.

The NOAEL was 100 ppm, approximately 3.0 mg/kg bw per day for males and 2.5 mg/kg bw per day for females, on the basis of histopathological findings (arteritis) at 200 ppm (Stebbins & Brooks, 2006).

Table 14. Incidence of chronic arterial inflammation in dogs given diets containing spinetoram for 1 year

Organ or tissue	Severity	Dietary concentration (ppm)							
		Males (n = 4 per group)				Females (n = 4 per group)			
		0	50	100	200	0	50	100	200
Epididymis	Very slight	1	0	0	0	—	—	—	—
Epididymisa	Slight	0	0	1	0	—	—	—	—
	Moderate	0	0	0	1	—	—	—	—
Larynx	Very slight	0	0	0	0	0	0	0	1
Thymus	Slight	0	0	0	0	0	0	0	1
Thyroid	Very slight	0	0	0	0	0	0	0	1
Urinary bladder	Very slight	0	0	0	0	0	0	0	1

From Stebbins & Brooks (2006)

[a] Chronic-active inflammation.

(b) Dermal administration

Rats

Groups of ten male and ten female Fischer 344 rats were exposed dermally to spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) at a dose of 0, 100, 500, or 1000 mg/kg bw per day at a semi-occluded skin test site for 6 h per day, 7 days per week, for 28 consecutive days. The test substance or vehicle (0.5% methylcellulose) was applied to an area of not less than 10% of the total body surface area on the back of the rat (from the scapulae to the hipbone and half way down the flank), which was clipped free of hair at least 24 h before initiation of dosing and on an as-needed basis during the study (approximately weekly). The exposure site was semi-occluded with gauze dressing and non-absorbent cotton. The rat was wrapped in an elastic bandage to hold the test material, gauze dressing and cotton in place.

The only treatment-related change observed was a minimal, localized, microscopic skin effect at the site of application, consisting of very slight or slight epidermal hyperplasia variably accompanied by very slight hyperkeratosis in the majority of males and females at 500 or 1000 mg/kg bw per day and in some males and females at 100 mg/kg bw per day. The Meeting considered that this was an adaptive response.

The NOAEL for systemic effects was 1000 mg/kg bw per day in males and females, the highest dose tested. The NOAEL for local effects on the skin was 1000 mg/kg bw per day, the highest dose tested (Thomas et al., 2005).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female Crl:CD1(ICR) mice were given diets containing spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L at a concentration of 0 (controls), 25, 80, 150 or 300 ppm (equal to 0, 3.0, 10.0, 18.8, and 37.5 mg/kg bw per day for males, and 0, 4.0, 12.8, 23.9, and 46.6 mg/kg bw per day for females) for up to 18 months. Doses were confirmed analytically before exposure and after approximately 4, 8, 12, and 16 months. A previous study of toxicity had shown spinetoram to be stable for at least 62 days at concentrations ranging from 0.0005% to 4%. Body weight, food consumption and compound intake (before exposure, weekly during the first 13 weeks of the study and then at approximately monthly intervals until study termination), ophthalmoscopic examination (at necropsy), haematology (at 12 months and 18 months) gross pathology and histopathology were evaluated.

Survival overall was ≥ 74% (in all groups receiving spinetoram and in the control group) and treatment had no effect on mortality.

On days 232–546, females at 300 ppm had decreases in mean body weights and mean body-weight gains that ranged from 0.3–5.4% and 4.3–15.4%, respectively, when compared with those of the controls. At study termination, the mean body weight and mean body-weight gain of females at 300 ppm were 1.3% and 5.8% lower than those of the controls, respectively. These differences were not statistically significant. There were no treatment-related effects on body weights or body-weight gains of females at 25, 80, or 150 ppm, or of males at any dose.

Males at 300 ppm had treatment-related increases in mean absolute (11.2%) and relative (12.1%) liver weights that were outside the range for historical controls in 18-month studies in mice recently conducted at this laboratory, but that were not statistically significant. There were no histopathological correlates to the increased liver weights. Treatment-related histopathological alterations occurred in the stomach, lungs, and epididymides of mice at 300 ppm (Table 15). The treatment-related alterations in the stomach consisted of an increase in the incidence and severity of hyperplasia of the glandular mucosa, with associated dilatation of mucosal glands and chronic

Table 15. Selected histopathology findings in mice fed diets containing spinetoram for 18 months

Finding	Severity	Dietary concentration (ppm)									
		Males					Females				
		0	25	80	150	300	0	25	80	150	300
Stomach											
Dilatation, mucosal gland, multifocal	Very slight	12	15	13	18	23*	14	18	14	15	26
	Slight	0	0	0	1	1	0	0	0	1	3
	Any severity	12	15	13	19	24*	14	18	14	16	29*
Hyperplasia, glandular mucosa, diffuse	Moderate	0	0	0	0	1	0	0	0	0	0
Hyperplasia, glandular mucosa, multifocal	Very slight	11	10	6	9	6	6	7	6	9	8
	Slight	3	4	3	2	9	3	5	3	4	8
	Moderate	0	0	0	0	0	0	0	0	0	3
	Any severity	14	14	9	11	15	9	12	9	13	19
Inflammation, chronic, glandular, submucosa, multifocal	Very slight	4	7	5	3	15*	9	8	7	4	12
	Slight	0	0	0	1	1	0	0	0	2	6*
	Any severity	4	7	5	4	16*	9	8	7	6	18
Lungs											
Aggregates of alveolar macrophages	Very slight	6	4	1	8	5	9	6	7	11	22*
	Slight	0	0	0	0	4	0	1	0	1	4
	Any severity	6	4	1	8	9	9	7	7	12	26*
Epididymides											
Vacuolization, epithelium, head	Very slight	17	14	18	17	23	—	—	—	—	—
	Slight	0	0	0	0	19*	—	—	—	—	—
	Any severity	17	14	18	17	42*	—	—	—	—	—

From Stebbins & Dryzga (2007)
*Statistically significant by the Yates chi-square test, alpha = 0.05, two-sided.

inflammation of the glandular submucosa. In general, the stomach alterations were most prominent in the region of the glandular mucosa near the limiting ridge, and lessened in the pyloric area. Females at 300 ppm had a treatment-related increase in the incidence of very slight or slight aggregates of alveolar macrophages in the lungs. In addition, four males at 300 ppm had slight aggregates of alveolar macrophages that were interpreted by the study authors to be treatment-related. Males at 300 ppm had a treatment-related increase in the incidence and severity of cytoplasmic vacuolization of epithelial cells lining the ducts in the head of the epididymides.

No significant increase in the incidence of tumours was observed in either male or female mice at any dose, indicating that spinetoram did not have carcinogenic potential under the conditions of this study.

The NOAEL was 150 ppm, equivalent to 18.8 mg/kg bw per day for males, and 23.9 mg/kg bw per day for females) on the basis of histopathological alterations at 300 ppm (Stebbins & Dryzga, 2007).

Rats

Groups of 65 male and 65 female Fischer 344 rats were fed diets containing spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor Lat a concentration of 0 (controls), 50,

250, 500, or 750 ppm for up to 2 years. The doses selected were based on the results of the 90-day study with spinetoram (Yano et al., 2005) in comparison with data from 90-day and long-term studies with a structural analogue (Bond et al., 1995). The time-weighted average doses ingested, based upon mean food consumption and mean body weight data were 0, 2.12, 10.8, 21.6, and 32.9 mg/kg bw per day for males and 0, 2.63, 13.2, 26.6, and 40.0 mg/kg bw per day for females, respectively. After 1 year of treatment, necropsy was performed for the assessment of toxicity in ten males and ten females per group, for the assessment of neuropathology in five males and five females per group, while the remaining 50 males and females per group were fed the respective diets for up to 2 years. The long-term study of neurotoxicity is reported separately in section 2.6.

Body weight, food consumption and compound intake (before exposure, weekly during the first 13 weeks of the study and then at approximately monthly intervals until study termination), ophthalmoscopic examination (at necropsy), haematology and clinical chemistry (at 12, 18 and 24 months), urine analysis (at 3, 6, 12, 18 and 24 months) gross pathology and histopathology (at 12 and 24 months) were evaluated.

Premixes were mixed periodically throughout the study based on stability data. Dietary concentrations were not adjusted for purity. Diets were prepared as a fixed percent of test material in rodent chow. All doses were confirmed analytically before exposure, and at approximately 4, 8, 12, 18, and 22 months. The homogeneity of the diets containing spinetoram at the lowest and highest concentrations was determined concurrently with dose confirmation. A previous study of toxicity had shown spinetoram to be stable for at least 62 days at concentrations ranging from 0.0005% to 4%.

Survival overall for males and females in all groups combined after 2 years was ≥ 54% and there was no treatment-related effect on mortality.

Effects attributed to ingestion of spinetoram for up to 2 years consisted of a marginal, but statistically significant reduction in body weight for males at 500 or 750 ppm (≤ 5%), statistically significant, increased relative and absolute heart weights in females at ≥ 250 ppm (approximately 11% at 750 ppm) and in relative heart weights in males at ≥ 500 ppm, in absolute and relative liver weights in females at ≥ 250 ppm, reaching statistical significance only at some doses. In terms of histopathology (Table 16), treatment-related effects were also noted as thyroid follicular-cell vacuolation in males and females at 500 or 750 ppm, and aggregates of macrophages-histiocytes in the mesenteric lymph nodes of males at 750 ppm and females at 500 or 750 ppm. Females at 500 or 750 ppm also had an increased incidence and/or severity of aggregates of macrophages-histiocytes in the mediastinal lymph nodes and Peyer patches of the ileum and the spleen, and decreased numbers of basophilic foci of altered hepatocytes. Furthermore, females at 750 ppm had an increased incidence of aggregates of alveolar macrophages in the lung, and retinal degeneration/vacuolation.

No treatment-related effects were observed in males or females given spinetoram at a dietary concentration of 50 or 250 ppm.

No significant increase in the incidence of tumours was observed in male or female rats at any dose, indicating that spinetoram did not have carcinogenic potential under the conditions of this study.

The NOAEL was 250 ppm, equivalent to 10.8 mg/kg bw per day for males and 13.2 mg/kg bw per day for females, on the basis of histopathological findings in the thyroid at higher doses (Yano et al., 2007).

2.4 Genotoxicity

An adequate range of studies was used to test spinetoram containing two different ratios of factor J to factor L for genotoxicity in vitro and in vivo. The results of these studies are summarized in Table 17.

In two different assays for reverse mutation in *Salmonella typhimurium* and *Escherichia coli* in vitro, spinetoram did not cause a positive increase in the mean number of revertants per plate with

Table 16. Selected histopathology findings in rats fed diets containing spinetoram for 2 years

Finding	Severity	Dietary concentration (ppm)									
		Male					Female				
		0	50	250	500	750	0	50	250	500	750
Thyroid gland (No. examined)		48	49	48	49	49	50	50	50	48	49
Vacuolation, follicular cell	Very slight	3	2	4	28*	8	0	0	0	40*	14*
	Slight	0	0	0	6*	29*	0	0	0	3	27*
Lymph node, mesenteric (No. examined)		50	50	49	50	50	50	50	50	50	50
Aggregates of macrophages-histiocytes, multifocal	Very slight	21	36*	35*	32*	5*	15	15	12	2*	3*
	Slight	21	9	9	15	15	28	32	29	28	16*
	Moderate	1	1	1	0	26*	5	3	5	19*	27*
Lymph node, mediastinal (No. examined)		48	50	49	49	50	48	50	48	50	50
Aggregates of macrophages-histiocytes, multifocal	Very slight	0	2	1	0	3	7	9	5	13	17*
Spleen (No. examined)		50	31	30	24	50	50	50	50	50	50
Aggregates of macrophages-histiocytes, white pulp; multifocal	Very slight	0	0	0	0	0	7	7	9	14	17*
Ileum (No. examined)		50	50	49	48	50	50	50	50	50	50
Aggregates of macrophages-histiocytes, Peyer patches	Focal, very slight	3	5	3	5	8	3	4	7	10	5
	Multifocal, very slight	1	3	3	1	3	2	2	1	8	7
Lung (No. examined)		49	50	50	50	50	50	50	50	50	50
Alveolar aggregates of macrophages-histiocytes, multifocal	Very slight	0	0	1	0	0	10	4	3	6	26*
Eye (No. examined)		49	28	19	20	50	50	50	50	50	50
Degeneration; retina; bilateral	Very slight	16	2	4	3	16	14	9	7	11	22
	Slight	0	0	0	0	1	0	1	0	0	12*
	Moderate	0	0	0	0	0	0	0	0	0	2
Vacuolation; retina; bilateral; multifocal	Very slight	0	0	0	0	0	0	0	0	0	9*

From Yano et al. (2007)
*Statistical difference from values for the controls by the Yates chi-squared test, alpha = 0.05, two sided.

any tester strain either in the presence or absence of metabolic activation (S9 fraction prepared from the liver of rats induced with Aroclor™ 1254). Spinetoram gave negative results in two assays for chromosomal aberration in rat lymphocytes in vitro. In two assays for forward gene mutation at the *Hgprt* locus in Chinese hamster ovary cells in vitro, spinetoram was not-mutagenic.

The genotoxic potential of spinetoram in vivo was evaluated by examining the incidence of micronucleated polychromatic erythrocytes (MN-PCE) in the bone marrow of mice. There were no statistically significant increases in the frequencies of MN-PCE in groups treated with spinetoram

Table 17. Results of studies of genotoxicity with spinetoram

End-point	Test system	Concentration or dose	Result	Reference
In vitro				
Reverse mutation	S. typhimurium strains TA98, TA100, TA1535 & TA1537	3.33–5000 µg/plate (+S9); 1.0–1000 µg/plate (–S9); in ethanol	Negative	Mecchi (2005)
	E. coli WP2urvA	33.3–5000 µg/plate (±S9); in ethanol		
Reverse mutation	S. typhimurium strains TA98, TA100, TA1535 & TA1537	10–2500 µg/plate (+S9); 1.0–1000 µg/plate (–S9); in ethanol	Negative	Mecchi (2007a)
	E. coli WP2uvvA	33.3–5000 µg/plate (±S9); in ethanol		
Chromosomal aberration	Rat lymphocytes	2.5–100 µg/ml (–S9); 5–100 µg/ml (+S9); in 1% DMSO	Negative	Charles et al. (2005a)
Chromosomal aberration	Rat lymphocytes	5–370 µg/ml –S9; 10–370 µg/ml +S9; in 1% DMSO	Negative	Schisler et al. (2007)
Forward mutation	Chinese hamster ovary cells (Hgprt locus)	5–200 µg/ml (±S9) Confirmatory assay: 10–100 µg/ml (–S9); 20–400 µg/ml (+S9); in 1% DMSO	Negative	Siedel et al. (2005)
Forward mutation	Chinese hamster ovary cells (Hgprt locus)	10–320 µg/ml (±S9) Confirmatory assay: 10–160 µg/ml (–S9); 20–320 µg/ml (+S9); in 1% DMSO	Negative	Schisler & Kleinert (2007)
In vivo				
Micronucleus formation	Male mouse bone-marrow polychromatic erythrocytes	500, 1000, 2000 mg/kg bw per day by oral gavage (two doses, 24 h interval); in 0.5% Methocel	Negative	Charles et al. (2005b)

DMSO, dimethyl sulfoxide; S9, 9000 × *g* supernatant from livers of male rats.

when compared with the negative controls. There were no statistically significant differences in the percentage of PCE in groups treated with spinetoram.

2.5 Reproductive toxicity

(a) Multigeneration study

Groups of 27 male and 27 female Crl:CD(SD) rats were fed diets providing spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) at a dose of 0, 3, 10, or 75°mg/kg bw per day for approximately 10 weeks before breeding, and continuing through breeding, gestation, and lactation for two generations. Statements of compliance with GLP and QA were provided. In-life parameters measured included clinical observations, food consumption, body weights, estrous cyclicity, thyroid-hormone analyses, reproductive performance, pup survival, pup body weights, and puberty onset. In addition, evaluations carried out post mortem included gross pathology, histopathology, organ weights, oocyte quantitation and sperm count, motility and morphology in adults, and gross pathology and organ weights in weanlings.

The overall actual mean concentrations of spinetoram in the diets fed to the rats during the study were 97.1%, 95.3%, and 93.9% of the target concentrations for males and 107%, 103%, and 92.0% of the target concentrations for females at 3, 10, and 75 mg/kg bw per day, respectively.

At a dose of 75°mg/kg bw per day, very slight to slight cytoplasmic vacuolation of the thyroid follicular epithelial cells was observed in adult males and females of both generations (Table 18).

However, these changes were not accompanied by any consistent, treatment-related changes in thyroid hormone levels (T_3, T_4 or TSH), although there were significant differences in some dose groups of either the parental (F_0) or the F_1 generation in one or other of the hormones. Absolute and relative liver weights were statistically significantly increased in the F_1 adult males and females at the highest dose, but there were no corresponding histopathological changes. In the kidneys of some parental (F_0) or F_1 females and occasional F_1 males at 75 mg/kg bw per day, there was a minor, subtle, treatment-related change that consisted of a very slight increase in the amounts of a light yellow-brown, pigmented material (probable lipofuscin-like substance) usually within a vacuole, in the proximal tubular epithelial cells at occasional foci. Neither the changes in liver weight nor the histopathological changes in the kidney were considered to be adverse.

Among females at 75 mg/kg bw per day, three F_0 and three F_1 females had complications of parturition (dystocia), in most cases the protracted delivery of pups over several days. These

Table 18. Incidence of histopathology findings in the parental generation (F0) in a study of reproductive toxicity in rats fed diets containing spinetoram

Finding	Dose (mg/kg bw per day)							
	Male				Female			
	0	3	10	75	0	3	10	75
Thyroid gland (No. examined)	27	26	26	27	27	27	27	27
Vacuolization, cytoplasmic, follicular cell:								
Very slight	0	0	0	4	0	0	0	10
Slight	0	0	0	22	0	0	0	14
Kidney (No. examined)	27	3	1	27	27	27	27	27
Pigment, increased, proximal tubule, multifocal:								
Very slight	0	0	0	0	0	0	0	9

From Carney et al. (2006)

Table 19. Incidence of histopathology findings in the F1 generation in a study of reproductive toxicity in rats fed diets containing spinetoram

Finding	Dose (mg/kg bw per day)							
	Male				Female			
	0	3	10	75	0	3	10	75
Thyroid gland (No. examined)	26	27	27	27	27	27	27	27
Vacuolization, cytoplasmic, follicular cell, diffuse:								
Slight	0	0	0	22	0	0	0	18
Kidney (No. examined)	27	27	27	27	27	27	27	27
Pigment, increased, proximal tubule, multifocal:								
Very slight	0	0	0	2	1	0	0	11

From Carney et al. (2006)

females also exhibited clinical signs (e.g. postpartum vulvar discharge, pale skin/mucous membranes, perinasal/perineal soiling), had reduced body weights and food consumption during lactation, and associated decreases in the survival and body weight of their pups. Two of these females were killed in a moribund condition as a secondary consequence of dystocia. Effects in the remaining litters of dams at the highest dose were limited to slightly decreased survival during gestation and an associated slight increase in post-implantation loss, although only the reduction in survival during gestation in the F_1 generation reached statistical significance. Overall, the effects at 75 mg/kg bw per day appeared to be maternally-mediated and restricted to the process of parturition. There were no effects on any parameter of reproductive performance or offspring growth and survival at 3 and 10 mg/kg bw per day, nor were there any reproductive effects in males at any dose.

The NOAEL for parental, reproductive and offspring toxicity was 10 mg/kg bw per day on the basis of slight thyroid vacuolation in adult males and females, dystocia in females at 75 mg/kg bw per day and decreased survival during gestation in pups at this dose (Carney et al., 2006).

(b) Developmental toxicity

Rats

In a preliminary evaluation of the maternal toxicity and embryo/fetal lethality potential of spinetoram in rats, groups of seven or eight time-mated CD rats were given spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) at targeted doses of 0, 50, 150 or 300 mg/kg bw per day by gavage in aqueous 0.5% methylcellulose (dose volume, 4 ml/kg bw; adjusted daily on the basis of individual body weights) on days 6 to 20 of gestation. In-life parameters evaluated for all groups included clinical observations, body weight, body-weight gain, and food consumption. On day 21 of gestation, all surviving rats were killed and examined for gross pathological alterations. Liver and kidney weights were recorded, as were the number of corpora lutea, implantations, resorptions, and live/dead fetuses.

At 300 mg/kg bw per day, spinetoram caused slight decreases in maternal body-weight gain early in the treatment period, although this was not statistically significant. There were no effects on any other parameters of maternal toxicity at this or lower doses. There were no treatment-related observations relating to gross pathology, nor any effects on reproductive parameters. There were no effects on any measure of embryo/fetal toxicity at any dose (Carney et al., 2005c).

The preliminary study was followed by the main study in which groups of 26 time-mated female CD rats were given spinetoram (suspended in 0.5% methylcellulose) at targeted doses of 0, 30, 100, or 300 mg/kg bw per day by oral gavage on days 6 to 20 of gestation. In-life maternal study parameters measured included clinical observations, body weight, body-weight gain, and food consumption. On day 21 of gestation, all rats were killed and examined for alterations in gross pathology. Liver, kidneys, and weights of the gravid uterine were recorded, together with the number of corpora lutea, uterine implantations, resorptions, and live/dead fetuses. All fetuses were weighed, sexed, and examined for external alterations. Approximately half of the fetuses were examined for visceral alterations while skeletal examinations were conducted on the remaining fetuses.

There was a statistically significant reduction in maternal body-weight gain and food consumption at 300 mg/kg bw per day (body weight gain was 43.5% less than that of the controls during days 6–9 of gestation). There was also a slight, though not statistically significant, decrease in body-weight gain during days 9–12 of gestation. As a result, body-weight gain over the dosing period (days 6–20 of gestation) in females receiving spinetoram at a dose of 300 mg/kg bw per day was decreased non-significantly by approximately 8% when compared with that of the controls.

No treatment-related embryo/fetal toxicity or teratogenicity was observed at doses of up to and including 300 mg/kg bw per day, There were no treatment-related fetal skeletal malformations identified in any of the treated groups when compared with the controls. At 300 mg/kg bw per day there was one litter with extra thoracic vertebrae, thoracic centra, thoracic rib, and sternebrae (three fetuses with the same anomalies), and one fetus with class II wavy ribs. The fetal findings of extra thoracic vertebrae, thoracic centra, thoracic rib, and sternebrae that occurred in the three fetuses are most likely to be due to a genetic effect, as they were limited to one litter, and no associated axial skeleton segmentation anomalies were observed.

The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of decreased maternal body-weight gain, and the NOAEL for developmental toxicity was 300 mg/kg bw per day, the highest dose tested (Carney et al., 2005a).

Rabbits

In a preliminary evaluation of the maternal toxicity and embryonal/fetal lethality of spinetoram in rabbits, groups of seven time-mated female New Zealand White rabbits were given spinetoram purity, 83.0%; 62% factor J and 21.0% factor L) at targeted doses of 0, 6.4, 15.7, 30 or 64 mg/kg bw per day by gavage in aqueous 0.5% methylcellulose (dose volume, 4 ml/kg bw; adjusted daily on the basis of individual body weights) on days 7 to 27 of gestation. Additional groups receiving spinetoram at 0, 100 or 150 mg/kg bw per day were subsequently evaluated in order to establish a better estimate of the maximum tolerated dose.

At 150 and 100 mg/kg bw per day, excessive maternal toxicity was evident as decreased food consumption, decreased faecal output, and decreased body weight during the treatment period. Owing to severe inanition and subsequent weight loss, all rabbits from these groups were killed by day 15 of gestation with no further data collection. At 64 mg/kg bw per day, food consumption was decreased, though this was rarely statistically significant. There was a decrease in faecal output in some of the rabbits at the highest dose. There were no effects on organ weights, gross pathology, or reproductive parameters in any of the rabbits surviving to scheduled necropsy (Carney et al., 2005b).

The preliminary study was followed by a main study studying which groups of 25–26 time-mated female New Zealand White rabbits were given spinetoram at targeted doses of 0, 2.5, 10, or 60 mg/kg bw per day by gavage on days 7 to 27 of gestation. In-life parameters evaluated for all groups included clinical observations, body weight, body-weight gain, and food consumption. On day 28 of gestation, all surviving rabbits were killed and examined for alterations in gross pathology and changes in weights of the liver, kidney, and gravid uterine. The number of corpora lutea, uterine implantations, resorptions and live/dead fetuses were determined. All fetuses were weighed, sexed and examined for external, visceral and skeletal alterations. The internal structures of the head were examined by serial sectioning of approximately one-half of the fetuses in each litter.

Treatment-related decreases in food consumption, and body weight gain, particularly during the earlier part of gestation (Table 20), faecal output, and statistically significant increases in mean absolute and relative liver weights were observed at rabbits at 60 mg/kg bw per day (Table 21).

In addition, one dam at 60 mg/kg bw per day was killed on day 21 of gestation due to inanition and subsequent weight loss that were interpreted by the study authors to be treatment-related. There were no maternal effects at the lower doses, and no signs of developmental toxicity at any dose.

The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of decreased food consumption, faecal output, and body-weight gain in females at 60 mg/kg bw per day. The NOAEL for developmental toxicity was 60 mg/kg bw per day, the highest dose tested (Carney et al., 2005d).

Table 20. Body-weight gains during gestation in a study of developmental toxicity in female rabbits given spinetoram by gavage

Time-point (days of gestation)	Mean body-weight gain (g)			
	Dose (mg/kg bw per day)			
	0	2.5	10	60
Days 7–10	20.9	16.6	34.3	–17.7[a]
Days 10–13	50.0	59.9	47.8	30.2
Days 13–16	82.2	71.7	79.9	11.5[a]
Days 16–20	29.7	46.7	31.7	14.2
Days 7–28	294.3	327.4	355.7	205.6[b]

From Carney et al. (2005d)

[a] Statistically different from mean for controls by Wilcoxon's test, alpha = 0.05.

[b] Statistically different from mean for controls by Dunnett's test, alpha = 0.05.

Table 21. Liver weights in a study of developmental toxicity in female rabbits given spinetoram by gavage

Weight	Dose (mg/kg bw per day)			
	0	2.5	10	60
Terminal body weight (g)	3478	3438	3540	3388
Liver (g)	75.27	76.14	81.24	83.25*
Liver (g/100)	2.164	2.218	2.299	2.459*

From Carney et al. (2005d)

* Statistically different from mean for controls by Dunnett's test, alpha = 0.05.

2.6 Special studies

(a) Acute neurotoxicity

Rats

In a study of acute neurotoxicity, groups of 10 male and 10 female F344/DuCrl rats were given spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) as a single oral dose at 0, 200, 630 or 2000 mg/kg bw by gavage. Body weights were recorded and a functional observational battery (FOB) and test for motor activity were conducted before exposure (baseline), on the day of dosing (day 1), and on day 8, and day 15. The FOB included hand-held and open-field observations as well as measurements of rectal temperature, grip performance, and landing-foot splay. Clinical observations were conducted on days 2, 3, and 4. At the end of the study, five males and females from the control group and from the group at the highest dose were perfused for histopathological evaluation of the central and peripheral nervous systems.

There were no treatment-related effects seen on body weight, FOB, motor activity, or on neuropathological evaluation at any time.

The NOAEL for acute neurotoxicity in male and female F344/DuCrl rats was 2000 mg/kg bw, the highest dose tested (Maurissen, 2005).

(b) Short-term study of neurotoxicity

As part of a long-term study toxicity and carcinogenicity study, a 1-year study of neurotoxicity was conducted in groups of 10 male and 10 female Fischer 344 rats given diets containing spinetoram at a concentration of 0, 50, 250, 500, or 750 ppm (equal to 0, 2.4, 12.0, 24.4, and 36.7 mg/kg bw per day in males and 0, 2.9, 14.7, 29.6, and 44.3 mg/kg bw per day in females). An automated test of motor activity, a FOB, determinations of grip performance, rectal temperature, and landing-foot splay evaluations were carried out before exposure, and after 1, 3, 6, 9, and 12 months. After 12 months (1 year) of exposure, five males and five females per group were perfused, and tissues from the central and peripheral nervous system of rats in the control group and rats at the highest dose were submitted for neuropathological examination.

No treatment-related effects were seen on grip performance, landing-foot splay, rectal temperature, or motor activity at any time. For the ranked and categorical FOB, there were no observations that could be attributed to treatment. There were no treatment-related findings after gross or histopathological examination of the central or peripheral nervous system after 12 months of dietary exposure.

In summary, there were no effects of spinetoram on any parameter that would suggest a neurotoxic effect, and the NOAEL for neurotoxicity in male and female Fischer 344 rats was 750 ppm, equal to 36.7 mg/kg bw per day, the highest dose tested (Maurissen, 2007).

(c) Studies on metabolites

The metabolites of spinetoram are not predicted to be present in groundwater at concentrations of greater than 0.1 µg/l and are therefore not considered to be toxicologically relevant. Although most metabolites of spinetoram have not therefore been tested individually for toxicity in mammals, the plant metabolites *N*-formyl-factor J and *N*-formyl-factor L have been evaluated in a study of acute oral toxicity and an Ames test. In addition, the metabolism of *N*-formyl-factor J was evaluated in the rat.

(i) Metabolism of metabolites of spinetoram

The plant metabolite *N*-formyl-factor J was extensively metabolized in the F344 rat. Based on the faecal metabolite profile seen with this material in rats, it is estimated that 21–28% of the administered dose is converted to metabolites that may be common to those formed from the parent factor J (Rick et al., 2005).

(ii) Acute toxicity of metabolites of spinetoram

Both *N*-formyl-factor J and *N*-formyl-factor L were of low acute toxicity (LD_{50} > 5000 mg/kg bw) and were found to lack mutagenic potential. These findings are consistent with the toxicity profile observed with parent spinetoram (Lowe, 2007a and 2007b).

3. Observations in humans

The period of development of spinetoram as a commercial product has been too short for any information from medical surveillance of manufacturing plant personnel to become available. There have been no exposure incidents involving laboratory or field personnel working with spinetoram. There are no medical reports of alleged human health effects associated with spinetoram.

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[1] Most of the studies of toxicity were conducted with factor J and factor L in a ratio equal to 75 : 25. Some studies were repeated with factor J and factor L in the ratio of 85 : 15; this was done to demonstrate that the 85 : 15 ratio produces a toxicity profile that is essentially the same as that seen with the 75 : 25 ratio.

Comments

Biochemical aspects

The toxicokinetics and metabolism of the two insecticidally active factors in spinetoram, factor J and factor L, are quite similar. In rats, the factors were rapidly and extensively (≥ 70%) absorbed. Peak plasma concentrations of radiolabel were achieved within 2–4 h. Systemic bioavailability was at least 26–29% for factor J and 39–57% for factor L. The factors were extensively distributed in the tissues, with highest concentrations in the gastrointestinal tract, fat, carcass and the liver. Excretion was primarily via the faeces (85%), mainly as metabolites, with only 3–4% of the administered dose excreted in the urine. Most of the administered dose was recovered within 24 h. The plasma half-lives of radiolabelled factor J and factor L were 4–11 h and 8–24 h, respectively. Very little radiolabel remained in the carcass after 7 days: 0.6–1.4% with factor J and 3–7% with factor L. Pre-treatment of rats with a low dose of either factor for 14 days did not affect the subsequent absorption and excretion of the respective factor.

Both factor J and factor L were extensively metabolized. The major metabolic pathway was glutathione conjugation, either of the parent, or of the products of *N*-demethylation, *O*-deethylation and deglycosylation of each factor, as well as hydroxylation of parent factor J. The aglycone of factor L was also subject to sulfate and glucuronide conjugation. The major metabolite was the cysteine conjugate of the parent factor.

Toxicological aspects[1]

Spinetoram was of low acute toxicity in rats: oral LD_{50} > 5000 mg/kg bw; dermal LD_{50} > 5000 mg/kg bw; and 4-h inhalational LC_{50} > 4.44 mg/l. There was no mortality at limit doses of 5000 mg/kg bw and 4.4 mg/l, respectively. Spinetoram is not a skin or eye irritan**t.**

In a local lymph node assay in BALB/c mice, spinetoram was shown to be a moderate skin sensitizer, while in a second assay in CBA/J mice (the recommended strain for this assay according to OECD TG 429 guidelines), spinetoram was not a skin sensitizer.

A range of effects was observed in short- and long-term studies with repeated dosing, and the effects were broadly similar in mice, rats and dogs. In short-term studies in mice, rats and dogs, cytoplasmic vacuolation of parenchymal cells, epithelial cells, macrophages and fibroblasts of a variety of tissues was observed, with some degeneration of muscle. There was also an increase in the incidence and/or severity of aggregates of macrophages/histiocytes in the lymphoid structures of numerous tissues. In mice, the NOAEL was 150 ppm, equal to 24.5 mg/kg bw per day, in a 28-day study. The NOAEL was 50 ppm, equal to 7.5 mg/kg bw per day, in a 90-day study in which there was also slight splenic extramedullary haematopoiesis in females at the lowest-observed-adverse-effect level (LOAEL). In rats, the NOAEL was 500 ppm, equal to 48 mg/kg bw per day, in a 28-day study in which there was vacuolation of the thyroid follicular epithelium and the renal tubular epithelium at the LOAEL. In three 90-day studies in which rats were exposed to spinetoram at two different ratios of factor J to factor L (75 : 25 and 85 : 15), the overall NOAEL was 10 mg/kg bw per day, the factor ratio having little effect on sensitivity. There was also an increase in reticulocyte and leukocyte counts at the LOAEL in one of these studies. In beagle dogs, the NOAEL was 200 ppm, equal to 5.9 mg/kg bw per day, in a 28-day study. In addition to vacuolation of numerous tissues, there was extramedullary splenic haematopoiesis at the LOAEL. In a 90-day study, the NOAEL was 150 ppm, equal to 5.0 mg/kg bw per day. Arteritis or perivascular inflammation and extramedullary haematopoiesis were also observed at the LOAEL in this study. The NOAEL in a 1-year study was 100 ppm, equal to 2.5 mg/kg bw per day, on the basis of arteritis, accompanied by necrosis of the arterial walls at the LOAEL of 200 ppm. The incidence of arteritis in the group receiving spinetoram at 200 ppm was low (one out of four males and one out of four females), and may have reflected the normal background incidence of lesions often seen in beagle

dogs; however, the fact that more severe effects that were considered to be treatment-related were noted in dogs given spinetoram at 300 or 900 ppm for 90 days suggested that these changes in the 1-year study may be treatment-related. The overall NOAEL was 5 mg/kg bw per day in dogs.

In long-term studies in rats and mice, tissue vacuolation was again commonly observed at doses at and above the LOAEL. In an 18-month study in mice, the NOAEL was 150 ppm, equal to 18.8 mg/kg bw per day, on the basis of histopathological changes in the stomach, lungs and epididymides at the LOAEL. In addition to cytoplasmic vacuolation of the epithelium of the ducts lining the head of the epididymides and aggregates of alveolar macrophages in the lungs, hyperplasia and inflammation of the glandular mucosa of the stomach, with dilatation of the mucosal glands were also observed. In a 2-year study in rats, the NOAEL was 250 ppm, equivalent to 10.8 mg/kg bw per day.

Selected tissues from short-term studies of toxicity with spinetoram and with the structurally related compound spinosad in rats (both compounds) and in mice (spinosad only) were examined by electron microscopy. Vacuolation was shown to be associated with cytoplasmic lamellar inclusion bodies, reflecting dysregulation of lysosomal storage (i.e. phospholipidosis). While such effects may arise through a variety of mechanisms that prevent degradation of cell constituents usually processed in the lysosomes, it is most likely that spinetoram acts through a physicochemical mechanism associated with its cationic amphiphilic structure, in common with other such compounds.

In long-term studies of toxicity and carcinogenicity, there was no evidence of treatment-related tumourigenicity in rats or mice. The Meeting concluded that spinetoram was not carcinogenic.

Spinetoram gave negative results in an adequate range of studies of genotoxicity in vitro and in vivo. The Meeting concluded that spinetoram had no genotoxic potential.

On the basis of the absence of carcinogenicity and genotoxicity, the Meeting concluded that spinetoram is unlikely to pose a carcinogenic risk to humans

The reproductive effects of spinetoram have been investigated in a two-generation study in rats. Cytoplasmic vacuolation of thyroid follicular epithelial cells was observed in adults of both generations at the highest dose (75 mg/kg bw per day). Among females at this dose, three parental (F_0) and three F_1 females had complications of parturition (dystocia), in most cases evidenced by the protracted delivery of pups over several days. These females also exhibited clinical signs (e.g., postpartum vulvar discharge, pale skin/mucous membranes, perinasal/perineal soiling), had reduced body weights and food consumption during lactation, and associated decreases in survival and body weight of their pups. The dystocia occurred in a few females (about 13%) at the highest dose of 75 mg/kg bw per day. A similar effect (in up to about 24% of litters) was seen with spinosad at a higher dose of 100 mg/kg bw per day. For both substances, the NOAEL for this effect was 10 mg/kg bw per day, which was also the NOAEL for maternal toxicity, For females at the highest dose without dystocia, gestational survival was slightly decreased, with an associated increase in postimplantation loss. No other measures of reproductive performance were affected in either males or females. The NOAELs for parental, reproductive and offspring toxicity were 10 mg/kg bw per day on the basis of slight thyroid vacuolation in adult males and females, dystocia in females and decreased gestation survival in pups at 75 mg/kg bw per day

The developmental toxicity of spinetoram had been investigated in rats and rabbits. In rats, maternal body weight and food consumption were reduced at 300 mg/kg bw per day, with a NOAEL of 100 mg/kg bw per day. There was no treatment-related embryo/fetal toxicity or teratogenicity at doses up to and including 300 mg/kg bw per day. The NOAEL for developmental toxicity was 300 mg/kg bw per day, the highest dose tested.

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[2] Marginal differences out of concurrent controls but within the range for historical controls.

In a preliminary study of developmental toxicity in rabbits, dams given doses of 150 or 100 mg/kg bw per day showed decreased food consumption, decreased faecal output, and decreased body-weight gain from the beginning of the treatment period. No other clinical findings were present in these two groups. The effect on body weight and faecal output, which were associated with a marked and consistent decrease in food consumption, were most likely a consequence of local irritation of the gastrointestinal tract. Owing to severe inanition and subsequent weight loss, all rabbits from these groups were killed by day 15 of gestation with no further data collection.

In the main study of developmental toxicity in rabbits, treatment with spinetoram resulted in decreases in food consumption, faecal output, and body-weight gain, and increased mean absolute and relative liver weights at a dose of 60 mg/kg bw per day. In addition, one dam at 60 mg/kg bw per day was killed on day 21 of gestation owing to inanition and subsequent weight loss, considered to be treatment-related. There were no signs of developmental toxicity at any dose. The NOAEL for maternal toxicity was 10 mg/kg bw per day. The NOAEL for developmental toxicity was 60 mg/kg bw per day, the highest dose tested.

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

Neurotoxicity was investigated in rats given single doses of up to 2000 mg/kg bw, or repeated doses of up to 750 ppm (36.7 mg/kg bw per day) for 12 months. Comprehensive behavioural and histopathological investigations revealed no evidence of neurotoxicity.

The plant metabolites *N*-formyl-factor J and *N*-formyl-XDE-1175-L were evaluated in a test for acute oral toxicity and in an Ames test for genotoxicity. Both metabolites were of low acute oral toxicity (LD_{50} > 5000 mg/kg bw) and gave negative results in the Ames test.

The development of spinetoram as a commercial product had been too short for any information from medical surveillance of manufacturing-plant personnel to be available. There were no documented cases of intoxication or of any other clinical effects associated with its use.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) 0–0.05 mg/kg bw based on an overall NOAEL of 5.0 mg/kg bw per day, identified on the basis of arteritis, accompanied by necrosis of the arterial walls in the affected organ(s), in studies of toxicity in dogs, and with a safety factor of 100. Although arteritis was observed only in some dogs, at an incidence that was within the range for historical controls, the incidence of arteritis at the LOAEL was greater in the concurrent controls and clear effects were found at higher doses in another study. Additionally, the structurally related compound spinosad had also been observed to cause arteritis in dogs given spinosad for 1 year, at doses not dissimilar to the LOAEL for the present study. Hence, the Meeting concluded that while there was some uncertainty as to the toxicological significance of the finding of arteritis at the LOAEL for spinetoram, use of the overall NOAEL from studies of toxicity in dogs as a basis for establishing the ADI was scientifically justified.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for spinetoram on the basis of its low acute toxicity, the absence of neurotoxic potential and of developmental or any other effects of relevance for acute exposure in studies of longer duration. Effects on gestational survival of pups observed in the multigeneration study in rats were most likely to be secondary to maternal toxicity, which was not a consequence of acute exposure.

[3] Recommended strain.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month combined toxicity and carcinogenicity[a]	Toxicity	150 ppm, equal to 18.8 mg/kg bw per day	300 ppm, equal to 37.5 mg/kg bw per day[c]
		Carcinogenicity	300 ppm, equal to 37.5 mg/kg bw per day[c]	—
Rat	2-year combined study of toxicity and carcinogenicity[a]	Toxicity	250 ppm, 10.8 mg/kg bw per day	500 ppm, equal to 21.6 mg/kg bw per day
		Carcinogenicity	750 ppm, equal to 32.9 mg/kg bw per day[c]	—
	Two-generation study[a]	Parental	10 mg/kg bw per day	75 mg/kg bw per day[c]
		Offspring toxicity	10 mg/kg bw per day	75 mg/kg bw per day[c]
		Reproductive toxicity	10 mg/kg bw per day	75 mg/kg bw per day[c]
	Developmental toxicity[b]	Maternal toxicity	100 mg/kg bw per day	300 mg/kg bw per day[c]
		Foetotoxicity	300 mg/kg bw per day[c]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	60 mg/kg bw per day[c]
		Foetotoxicity	60 mg/kg bw per day[c]	—
Dog	Oral 90-day and 1-year studies	Toxicity	150 ppm, equal to 5.0 mg/kg bw per day	200 ppm, equal to 5.4 mg/kg bw per day)[2]

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

Estimate of acceptable daily intake for humans

0–0.05 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 exposures

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

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid (tmax 2–4 h) and extensive (> 70%). Systemic bioavailability of factor J (26–29%) < factor L (39–57%)
Distribution	Rapidly and extensive. Highest concentrations of radioactivity in the gastrointestinal tract, followed by fat, carcass and liver
Potential for accumulation	Tissue and carcass concentrations low after 7 days (0.6–1.4% of administered dose).

Rate and extent of excretion	Rapidly excreted, plasma half-lives 4–24 h; 85% of dose in faeces, mainly as metabolites; 3–4% in urine, mostly in first 24 h
Metabolism in animals	Extensively metabolized, primarily by glutathione conjugation of parent and products of phase-one metabolism. Some sulfate and glucuronide conjugation of aglycone of factor L
Toxicologically significant compounds (animals, plants and environment)	Spinetoram, comprising factors J and L

Acute toxicity

Rat, LD50, oral	> 5000 mg/kg bw
Rat, LD50, dermal	> 5000 mg/kg bw
Rat, LC50, inhalation	> 5.44 mg/l for 4 h (nose only)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Transient irritation
Mouse, dermal sensitization	Not sensitizing (local lymph node assay in CBA/J mice)[3]

Short-term studies of toxicity

Target/critical effect	Mice, rats, dogs: vacuolation of macrophages in a wide range of lymphoid tissues within numerous organs and aggregates of macrophages/histiocytes in a number of tissues, non-regenerative anaemia, arteritis (dogs)
Lowest relevant oral NOAEL	5.0 mg/kg bw per day (90-day and 1-year study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (28-day study in rats, highest dose tested)
Lowest relevant inhalation NOAEL	No data

Genotoxicity

	Negative in vitro and in vivo

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Mice, rats: vacuolation of cells (thyroid in rats; epididymes in mice) and increases in aggregates of macrophages/histiocytes in lymphoid tissues in numerous organs, hyperplasia of the glandular mucosa of the stomach and inflammation of the glandular submucosa (mice)
Lowest relevant NOAEL	2-year study, rat: 10.8 mg/kg bw per day
Carcinogenicity	Not carcinogenic

Reproductive toxicity

Reproduction target/critical effect	Dystocia (difficulty in delivery), decrease in gestation survival of pups.
Lowest relevant reproductive NOAEL	10 mg/kg bw per day (rats)
Developmental target/critical effect	None
Lowest relevant developmental NOAEL	60 mg/kg bw per day (rabbit; highest dose tested)

Neurotoxicity/delayed neurotoxicity

Acute neurotoxicity and short-term studies of neurotoxicity	No indications of neurotoxicity in single- or repeat-dose studies

Medical data

	No data available on manufacturing-plant personnel (production-scale manufacturing has yet to start). No reports of adverse health effects in exposed subjects.

Summary

	Value	Study	Safety factor
ADI	0–0.05 kg bw	Dog, 90-day and 1-year study	100
ARfD	Unnecessary	—	—

Factor J, XDE-175-J
Factor L, XDE-175-L

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SPIROTETRAMAT

First draft prepared by
D. Kanungo[1] & A. Moretto[2]

[1] Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India, New Delhi, India; and
[2] Department of Environmental and Occupational Health, University of Milan, International Centre for Pesticide and Health Risk Prevention, Luigi Sacco Hospital, Milan, Italy

Explanation

Spirotetramat is the ISO approved name for *cis*-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro[4,5]dec-3-en-2-one (IUPAC). The CAS No. for spirotetramat is 203313-25-1. Spirotetramat belongs to the chemical class of ketoenols, subclass tetramic acid derivatives, and is intended for use as an insecticide on a range of agricultural crops. The pesticidal mechanism of action is disruption of lipogenesis as a result of inhibition of acetyl CoA carboxylase.

The JMPR has not previously evaluated spirotetramat. Spirotetramat was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR) at its 40th Session.

The impurity profiles of batches of spirotetramat used in studies of toxicity were variable. Some impurities were absent in the material used in long-term studies of toxicity and studies of genotoxicity, or were present at a low concentration. However, the results of studies with impurities indicated that this was not a critical issue in the toxicological evaluation. All critical studies complied with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

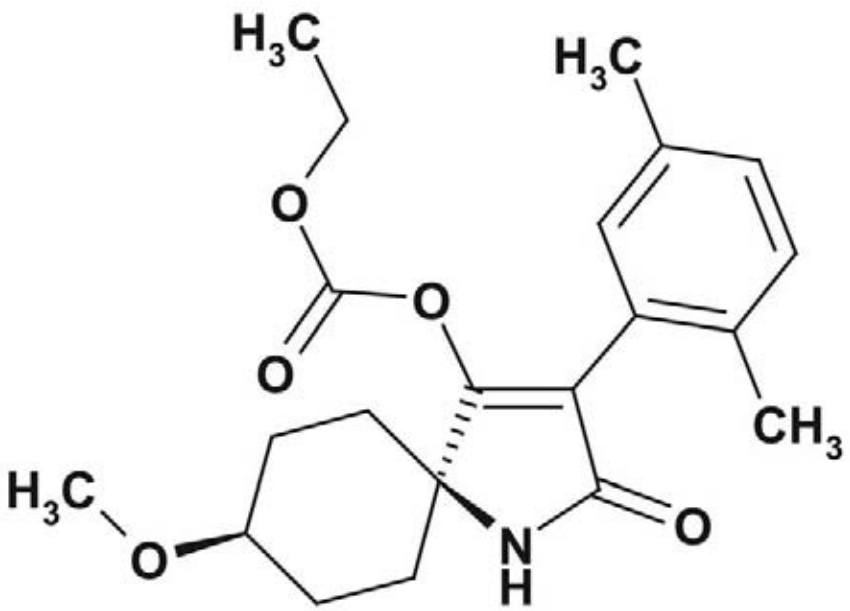

Figure 1. Chemical structure of spirotetramat

Rats

In a study of absorption, distribution, metabolism and excretion, groups of four male or four female rats (*Rattus norvegicus domesticus*, strain Wistar Hsd/Cpb:WU) were given a single dose of [azaspirodecenyl-3-^{14}C]spirotetramat (purity, > 99%; radiochemical specific activ-

ity, 100.2–99.1 μCi/mg, equal to 3.67–3.71 MBq/mg) at a target dose of 2 or 100 mg/kg bw by gavage in aqueous Tragacanth®. Two additional groups of four male or four female rats were pretreated for 14 days with non-radiolabelled spirotetramat at a dose of 2 mg/kg bw per day followed by a single dose of radiolabelled spirotetramat at 2 mg/kg bw. The active substance was radiolabelled with ^{14}C in the 3-position of the spirodecenyl ring of the molecule. The rats were aged 9 weeks (male) and 12–13 weeks (female) at study initiation. All rats were sacrificed 2 days after dosing.

Total radioactivity (including test substance and its metabolites) was determined in samples of plasma, excreta (urine and faeces) and in organs and tissues. Metabolism was investigated by high-performance liquid chromatography (HPLC), normal-phase thin-layer chromatography (TLC) and spectroscopic methods in selected samples of urine and faecal extracts.

Between 91.4% and 99.8% of the administered dose was recovered in the urine, faeces and organs and tissues at sacrifice. The recovery of radiolabel in the excreta, organs and tissues is summarized in Table 1.

[Azaspirodecenyl-3-^{14}C]Spirotetramat was very rapidly absorbed from the gastrointestinal tract of male and female rats in all test groups. The absorption commenced immediately after dosing. Values calculated for the absorption half-lives were in the range of 0.6–10 min. In this study, the minimal absorption rate in all tests was between 90% and 98% of the total radioactivity recovered, calculated from the values of the urine and the body without the gastrointestinal tract. No significant differences were seen between the lower, higher and repeated doses.

The maximum plasma concentration was reached for all dose groups within 0.09 to 2.03 h after administration (values calculated by pharmacokinetic modelling). From the maximum, the radioactivity concentrations in plasma declined steadily by several orders of magnitude within 48 h for all dose groups. The pharmacokinetic parameters (three-compartment modelling using the TOPFIT software program) are given in Table 2.

The results obtained from the experiments with the lower dose were nearly identical for males and females. Absorption of radiolabel was slightly slower in males than in females when comparing t_{max} values. Absorption was followed by a fast initial elimination phase and a moderate terminal elimination phase. The area under the curve [AUC (0–∞)] indicated a slightly higher systemic exposure for males than for females. The elimination rate constant was very similar for males and females. The mean residence time was low in males and slightly higher in females. The results obtained with the lower dose were in the same range after repeated and single doses.

At the higher dose, the C_{max} was significantly higher for males than for females. Compared with the lower dose, the plasma concentrations in males were proportional to the dose ratio with a slightly higher value for AUC (0–∞) in the test with the higher dose. The C_{max} was slightly lower for females at the higher dose than for females at the lower dose, while the value for AUC (0–∞) was proportional to the dose ratio. This indicated that the absorption process had not been (over) saturated at the respective highest dose. As with the lower dose, absorption was followed by a fast initial elimination phase and a moderate terminal elimination phase. The elimination rate constant was lower than for the lower dose and lower for males than for females. The mean residence time was in the same range as for the lower dose and was quite similar for males and females. Comparison of the absorption phases of the kinetic curves between the groups at the lower and higher dose showed a broader maximum for males at the higher dose. The maximum concentration (C_{max}) was reached slightly later at this dose and the following initial elimination phase was slightly longer than observed for the lower dose. The curves at the end of the terminal elimination phase were comparable. Plasma concentrations of radiolabel are summarized in Table 3.

The distribution of radioactive residues in the body was analysed at the time of sacrifice, 48 h after dosing, by measurement of the concentrations of radiolabel in the major organs and tissues. Less than 0.2% of the administered dose was detected in the body. The residues in all organs and

tissues at the time of sacrifice were low in all tests and sometimes below the limit of detection (LOD). The highest equivalent concentrations were detected in the liver (0.002–0.18 mg/kg) and kidney (0.001–0.11 mg/kg), the organs responsible for degradation and excretion. The recovery of radiolabel in organs and tissues of rats 48 h after oral administration of [azaspirodecenyl-3-^{14}C]spirotetramat is shown in Table 4.

Excretion was fast and almost complete within 24 h after dosing. There were no significant differences between males and females or between doses. Excretion was mainly renal and quite similar for males and females given either dose, either as a single dose or as repeated doses. About

Table 1. Recovery of radiolabel from rats killed 48 h after dosing with [azaspirodecenyl-3-^{14}C] spirotetramat

Sample	Recovery of radiolabel (% of administered dose)					
	Dose (mg/kg bw)					
	No pretreatment				Pretreatment[a]	
	2		100		2	2
	Male	Female	Male	Female	Male	Female
Urine	93.34	87.92	89.14	93.78	91.48	94.78
Faeces	5.11	3.34	10.51	2.98	6.59	1.78
Total excreted	98.45	91.26	99.65	96.76	98.07	96.56
Skin	0.004	0.070	0.015	0.014	0.011	0.024
Organs, total	0.019	0.058	0.112	0.021	0.067	0.036
Body, excluding gastrointestinal tract	0.023	0.129	0.126	0.035	0.078	0.060
Gastrointestinal tract	0.014	0.043	0.012	0.012	0.035	0.024
Total, body	0.038	0.171	0.138	0.047	0.113	0.083
Balance[b]	98.49	91.44	99.78	96.81	98.19	96.65
Recovery of radiolabel (% of total recovered radioactivity)						
Urine	94.79	96.06	89.34	96.86	93.23	98.06
Faeces	5.18	3.75	10.52	3.10	6.66	1.85
Total excreted	99.97	99.81	99.86	99.96	99.89	99.91
Skin	0.004	0.078	0.015	0.014	0.012	0.025
Organs, total	0.019	0.064	0.113	0.022	0.069	0.037
Body, excluding gastrointestinal tract	0.024	0.142	0.127	0.036	0.080	0.062
Gastrointestinal tract	0.014	0.047	0.012	0.013	0.036	0.025
Total, body	0.038	0.189	0.139	0.049	0.117	0.086
Normalization factor	1.015	1.101	1.002	1.034	1.022	1.036
Absorption rate	94.81	96.20	89.47	96.90	93.31	98.12

From Klempner (2006a)

[a] Fourteen doses of non-radiolabelled spirotetramat plus a single dose of radiolabelled spiriotetramat.

[b] Balance = total excreted + total retained in the body.

88–95% of the administered dose was eliminated in the urine and about 2–11% in the faeces. The concentration of radioactive residues in the organs and tissues were determined at sacrifice, 48 h after dosing. Negligible amounts of radiolabel were found in the carcass (including organs), showing that the elimination of compound-related radioactivity was nearly complete. No sex differences were observed in the amounts of residues in the organs and tissues. The time-course of excretion of radioactivity after oral administration of [azaspirodecenyl-3-^{14}C]spirotetramat is shown in Table 5.

Parent compound was not detected in the urine and faeces of rats in any of the tests. Two metabolites were prominent in all samples: the spirotetramat-enol, which was the major metabolite in urine and faeces of males and females in all tests, and the spirotetramat-desmethyl-enol. A sex-related difference in the ratio of the two main metabolites was observed in males and females in all

Table 2. Distribution and plasma kinetics of radiolabel in rats given [azaspirodecenyl-3-^{14}C] spirotetramat

Parameter	Units	Dose (mg/kg bw)					
		No pretreatment				Pretreatmenta	
		2	2	100	100	2	2
		Male	Female	Male	Female	Male	Female
C_{max} (model)	µg/g	4.41	4.15	210	117	5.21	2.98
t_{max} (model)	h	0.89	0.09	2.03	0.77	0.45	0.35
C_{max} (experiment)	µg/g	4.40	3.84	204	116	4.96	2.69
t_{max} (experiment)	h	1.00	0.17	1.50	0.66	0.66	0.66
$t_{1/2}$ a	h	< 0.01	< 0.01	0.17	0.06	0.10	0.07
$t_{1/2}$ e (1)	h	0.31	4.79	1.70	0.19	3.62	0.47
$t_{1/2}$ e (2)	h	20.1	29.7	17.5	27.2	92.7	13.2
t_{lag} a	h	< 0.01	0.08	0.06	0.05	0.03	0.03
AUC (0–∞)	µg/g × h	16.4	10.2	1380	451	14.6	7.64
K_1 e	1/h	246	258	0.98	3.53	5.61	8.53
CL/f	ml/min per kg	2.03	3.27	1.21	3.70	2.28	4.36
CLR	ml/min per kg	1.89	2.89	1.08	3.47	2.09	4.13
MRT	h	3.39	9.68	4.90	4.26	5.29	4.38
MRTabs	h	2.55	1.16	2.53	2.99	1.91	1.94
MRTdisp	h	0.84	8.52	2.37	1.27	3.38	2.44
Vss	l	0.10	1.67	0.17	0.28	0.46	0.64
Weighting function[b]	Number	g = 1	g = 1/y	g = 1	g = 1/y	g = 1	g = 1
Compartment[c]		3	3	3	3	3	3

From Klempner (2006a)

AUC, area under the curve of conentration–time; CL, clearance; CL/f, Total clearance of radioactivity from plasma; CLR, renal clearance radioactivity; C_{max}, maximum plasma concentration; K_{1e}, Elimination rate constant; MRT, mean residence time; $t_{1/2}$a, half-life of absorption; $t_{1/2}$ e (1), half-life of the elimination phase 1, equals initial elimination phase; $t_{1/2}$ e (2), half-life of the elimination phase 2, equals intermediate elimination phase; t_{laga}, lag between administration and the onset of absorption; t_{max}, time at which the maximum radioactivity concentration occurs in plasma after administration of extravascular dose; Vss, volume of distribution (apparent) of the radioactivity under steady-state conditions based on compound-related radio activity in plasma.

[a] Fourteen doses of non-radiolabelled spirotetramat plus a single dose of radiolabelled spirotetramat.

[b] For some individual data points, weighting factors were used for better curve-fitting.

[c] Three-compartment modelling using the TOPFIT software program.

tests, but the difference was greater in tests with the higher dose. The quantity of the major metabolite, spirotetramat-enol, was lower in males than in females. Conversely, the quantity of the second main metabolite, spirotetramat-desmethyl-enol, was higher in males than in females. The recovery of spirotetramat-enol ranged from 53% to 66% of the administered dose in males and from 81% to 87% in females. The recovery of spirotetramat-desmethyl-enol ranged from 25% to 37% of the dose in males and from 5% to 10% in females. The results for the single lower dose and repeated lower dose with pretreated rats were in the same range. Four more metabolites identified in the excreta were of minor importance. They ranged from 0.1% to 1.6% of the administered dose. They all were degradation products of the spirotetramat-enol. The first important metabolic reaction in males and females was the cleavage of the ester bond of the side-chain, yielding spirotetramat-enol, followed by demethylation of the methoxy group at the cyclohexyl ring. Cleavage of the molecule was not observed. All other metabolic reactions, such as conjugation of the spirotetramat-enol with glucuronic acid, hydroxylation in the pyrrolidine ring of spirotetramat-enol resulting in spirotetramat-ketohydroxy and oxidation of one of the methyl groups of the phenyl ring, were of minor importance. The proposed metabolic pathway of spirotetramat in male and female rats is shown in Figure 2. The study complied with GLP and a statement of quality assurance (QA) was provided (Klempner, 2006a).

A study of metabolism and pharmacokinetics was undertaken to investigate the depletion of residues of spirotetramat from the plasma, testes, liver and kidney, excretion in the urine and metabolism in groups of four male Wistar Hsd/Cpb:WU rats (aged 9 weeks) given a single dose of [azaspirodecenyl-3-^{14}C]spirotetramat (radiochemical purity, >98%) at 2 or 1000 mg/kg bw (three groups per

Table 3. Time-course of plasma concentrations of radiolabel in rats given oral doses of [azaspirodecenyl-3-^{14}C]spirotetramat

Timepoint (after dosing)	Equivalent concentration [µg/g] (measured values) (n = 4)					
	Dose (mg/kg bw)					
	No pretreatment				Pretreatmenta	
	2		100		2	2
	Male	Female	Male	Female	Male	Female
0.08	1.435	2.225	14.436	16.071	1.784	1.389
10.2 min	2.568	3.836	45.424	52.592	3.935	2.301
20 min	3.396	3.239	101.600	89.249	4.891	2.673
40 min	4.139	2.925	157.374	116.044	4.962	2.687
1 h	4.402	2.394	189.735	115.816	4.383	2.179
1.5 h	4.004	1.598	204.390	100.464	3.401	1.531
2 h	3.390	1.067	201.130	87.011	2.581	0.929
3 h	2.358	0.659	194.017	58.094	1.661	0.395
4 h	1.558	0.515	168.045	40.126	1.114	0.229
6 h	0.676	0.356	122.464	21.094	0.495	0.142
8 h	0.327	0.280	78.375	16.608	0.246	0.132
24 h	0.005	0.035	0.415	0.204	0.009	0.014
32 h	0.003	0.023	0.189	0.150	0.003	0.010
48 h	0.002	0.011	0.154	0.098	0.002	0.009

From Klempner (2006a)

[a] Fourteen doses of non-radiolabelled spirotetramat plus a single dose of radiolabelled spirotetramat.

dose) by oral gavage in aqueous Tragacanth®. The lower dose was selected to match the lower dose of spirotetramat administered in the previously described study of absorption, distribution, metabolism and excretion (Klempner, 2006a). Taking into account the findings from a mechanistic study, in which degenerative effects on round/elongating spermatids in the testes were found in male rats given more than 10 doses of spirotetramat at 1000 mg/kg bw per day; the higher dose selected was 1000 mg/kg bw (**Kennel, 2005**).

The rats were sacrificed 1 h, 8 h and 24 h after dosing. The total quantity of radiolabel, including parent compound and metabolites, was determined in samples of urine and faeces, as well as in the plasma, testes, liver and kidney at sacrifice. Investigations on metabolites were performed by determination of radiolabel in eluates from high-performance liquid chromatography (radio-HPLC) with selected samples of urine and plasma, and with extracts from the testes, liver and kidney.

Between 100.3% and 121.8% (lower dose) and 94.8% and 98.9% (higher dose) of the administered doses were recovered in the urine and faeces, and in organs and tissues at sacrifice. The slightly

Table 4. Recovery of radiolabel from tissues and organs of rats killed 48 h after oral administration of [azaspirodecenyl-3-^{14}C]spirotetramat

Tissue/organ	Equivalent concentration [µg/g][a] (n = 4)					
	Dose (mg/kg bw)					
	No pretreatment				Pretreatmentb	
	2	2	100	100	2	2
	Male	Female	Male	Female	Male	Female
Erythrocytes	0.0010	0.0013	0.0385	0.0250	0.0007	0.0007
Plasma	0.0011	0.0015	0.0703	0.0267	0.0009	0.0010
Spleen	0.0006	0.0009	0.0626	< LOD	0.0006	0.0006
Gastrointestinal tract	0.0024	0.0094	0.0809	0.0999	0.0035	0.0046
Liver	0.0076	0.0035	0.1792	0.0502	0.0094	0.0019
Kidney	0.0009	0.0040	0.1065	0.0609	0.0024	0.0027
Perirenal fat	< LOD	< LOD	< LOD	< LOD	0.0047	< LOD
Adrenal gland	< LOD	< LOD	< LOD	< LOD	0.0062	< LOD
Testes	0.0008	—	0.0622	—	0.0003	—
Ovaries	—	< LOD	—	< LOD	—	< LOD
Uterus	—	< LOD	—	< LOD	—	0.0015
Skeletal muscle	< LOD	0.0013	0.0377	< LOD	0.0006	< LOD
Bone (femur)	< LOD	0.0030	0.0855	0.0534	0.0009	< LOD
Heart	0.0006	0.0010	0.0332	0.0189	0.0006	0.0005
Lung	0.0005	0.0011	0.0327	0.0220	0.0006	0.0007
Brain	< LOD	0.0005	< LOD	< LOD	< LOD	< LOD
Thyroid gland	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Skin	0.0008	0.0060	0.0567	0.0529	0.0008	0.0022
Carcass	< LOD	0.0015	0.1649	0.0257	0.0009	0.0010

From Klempner (2006a)
< LOD, below the limit of detection.
[a] The concentrations are mean values for the four rats in each group.
[b] Fourteen doses of non-radiolabelled spirotetramat plus a single dose of radiolabelled spirotetramat.

higher recoveries obtained from two groups of rats at the lower dose were caused by the foamy consistence of the suspension of spirotetramat, which made it difficult to accurately measure the volume to be administered by gavage. The entire balances for the total amount of radiolabel detected in the urine, faeces, gastrointestinal tract plus faeces, skin and organs and tissues at sacrifice are shown in Table 7.

A summary of the experimental protocol is shown in Table 6.

Table 5. Time-course of excretion of radiolabel in the urine and faeces of rats given oral doses of [azaspirodecenyl-3-^{14}C]spirotetramat

Timepoint (hours after dosing)	Cumulative excretion of radiolabel (% of administered dose)					
	Dose (mg/kg bw)					
	No pretreatment				Pretreatmenta	
	2	2	100	100	2	2
	Male	Female	Male	Female	Male	Female
Urine						
4	34.48	36.50	17.95	55.19	40.65	56.35
8	77.48	45.10	50.87	78.95	77.77	57.55
12	89.95	[b]	[b]	[b]	[b]	[b]
24	92.96	85.68	88.32	93.03	90.86	93.16
48	93.34	87.92	89.14	93.78	91.48	94.78
Faeces						
24	4.89	2.30	9.95	2.79	5.94	1.44
48	5.11	3.34	10.51	2.98	6.59	1.78
Total excreted	98.45	91.27	99.64	96.76	98.08	96.56

From Klempner (2006a)

[a] Fourteen doses of non-radiolabelled spirotetramat plus a single dose of radiolabelled spirotetramat.

[b] No samples collected.

Table 6. Experimental protocol in a study of the metabolism and disposition of [azaspirodecenyl-3-^{14}C]spirotetramat in rats

Dose (mg/kg bw)	Time-point (h)	Comments
Single (2 mg/kg bw)	1	Urine collected at 0–1 h; blood, testes, liver, kidney, gastrointestinal. tract (+ contents), skin and carcass assessed at sacrifice.
	8	Urine collected at 0–1 and 4–8 h; blood, testes, liver, kidney, skin and carcass assessed at sacrifice.
	24	Urine collected at 0–1, 4–8 h, 8–24 h; blood, testes, liver, kidney, gastrointestinal tract (+ contents) skin and carcass assessed at sacrifice.
Single (1000 mg/kg bw)	1	Urine collected at 0–1 h; blood, testes, liver, kidney, skin and carcass assessed at sacrifice.
	8	Urine collected at 0–1 and 4–8 h; blood, testes, liver, kidney, skin, carcass assessed at sacrifice.
	24	Urine collected at 0–4, 4–8 h, 8–24 h; blood, testes, liver, kidney, gastrointestinal tract (+ contents) skin and carcass assessed at sacrifice.

From Klempner (2006b)

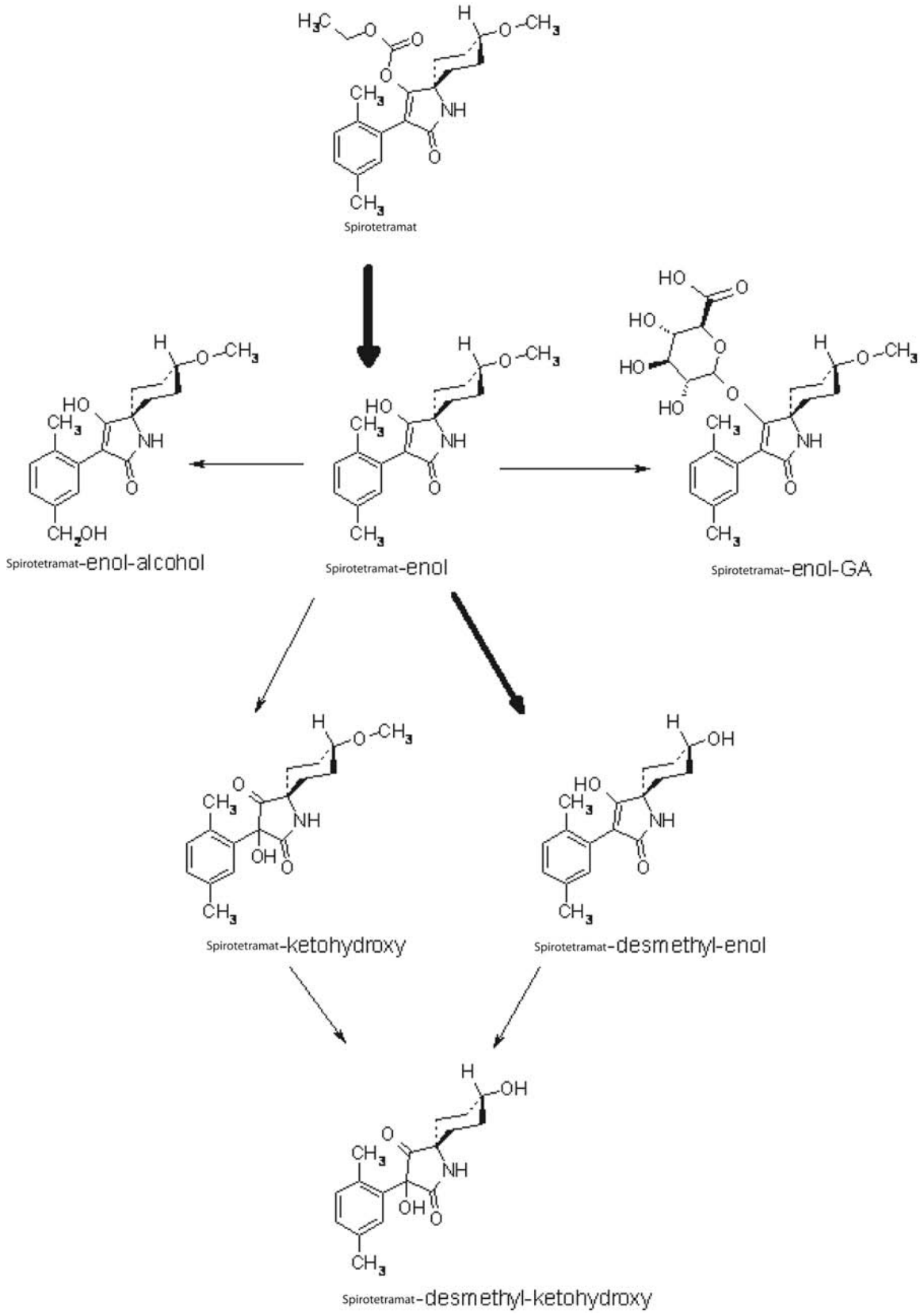

Figure 2. Proposed metabolic pathway of spirotetramat in rats

Urinary excretion was rapid in the groups receiving the lower dose, being almost complete within the first 24 h after administration. About 92% of the total recovered radiolabel (equivalent to 112% of the administered dose) was excreted via the urine. About 8% of the total recovered radiolabel (equivalent to 10% of the administered dose) was detected in faeces of rats given the lower dose, 24 h after dosing. Excretion was considerably slower in the rats at the higher dose than in those at the lower dose. Only 27% of the administered dose was excreted via the urine 24 h after dosing and 18% via the faeces, respectively. Most of the radiolabel was recovered from the gastrointestinal tract.

At sacrifice, the highest values for total radioactive residue (TRR) were measured in the organs and tissues sampled 1 h after administration (Table 8). Values declined rapidly to less than 0.05 mg/kg

Table 7. Balance of radioactivity in excreta, plasma, organs and tissues of male rats (n = 4) given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat

	Mean recovery of radiolabel (% of administered dose)					
	Dose (mg/kg bw)					
	2			1000		
Specific radioactivity [kBq/mg]	3670	3670	3670	7.34	7.34	7.34
Time-point (hours after dosing)	1 h	8 h	24 h	1 h	8 h	24 h
Urine						
0–1 h	12.5	-—	-—	1.6	-—	-—
0–4 h	-—	20.1	37.3	-—	4.9	6.0
4–8 h	-—	45.6	39.4	-—	10.9	9.4
8–24 h	-—	-—	35.3	-—	-—	11.4
Urine, total	12.5	65.7	111.9	1.6	15.8	26.8
Faeces	-—[a]	-—[a]	9.5	-—[a]	-—[a]	17.8
Blood						
Erythrocytes	1.4	0.3	0.003	0.3	0.2	0.1
Plasma	3.5	1.0	0.003	0.5	0.4	0.1
Organs						
Liver	22.9	9.2	0.067	1.2	0.9	0.4
Kidney	4.8	1.5	0.005	0.3	0.2	0.1
Testis	0.4	0.2	0.002	0.1	0.1	< 0.1
Organs, total	33.1	12.3	0.080	2.4	1.8	0.7
Skin	12.7	3.2	0.031	3.0	2.4	0.7
Carcass	28.3	10.6	0.097	5.9	6.1	1.8
Gastrointestinal tract	32.1	8.6	0.205	85.9	72.9	47.0
Total, body	106.2	34.6	0.413	97.1	83.1	50.2
Balance	118.7	100.3	121.8	98.7	98.9	94.8

From Klempner (2006b)

[a] For rats killed at 1 h and 8 h, the faeces were included with the gastrointestinal tract.

within 24 h in rats at the lower dose. The decline was slow after the higher dose; there was even a slight increase in testes and carcass from 1 h to 8 h after dosing.

In rats given the lower dose, parent compound was not detected in any sample or tissue. Spirotetramat-enol was the main metabolite in all samples. Spirotetramat-desmethyl-enol was the second most prominent metabolite in urine, plasma and testes, whereas in liver and kidney, spirotetramat-ketohydroxy was found to be the second most prominent metabolite. Spirotetramat-ketohydroxy was only present at trace concentrations in the plasma, testes and urine. Spirotetramat-enol-glucuronic acid (GA), spirotetramat-enol-alcohol and spirotetramat-desmethyl-ketohydroxy were detected at low concentrations and are therefore of minor importance. Twenty-four hours after dosing, residues in tissues and plasma were too low for quantification of metabolites. Identification rates were high, being in the range of 89–100%. The metabolic profiles for urine were in

Table 8. Total radioactive residues in organs and tissues of male rats (n = 4) given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat

Sample	Mean concentration of total radioactive residues (mg active substance equivalents/kg)					
	Dose (mg/kg bw)					
	2	2	2	1000	1000	1000
Specific radioactivity [KBq/mg]	3670	3670	3670	7.34	7.34	7.34
	1 h	8 h	24 h	1 h	8 h	24 h
Erythrocytes	1.723	0.412	0.003	199.2	147.9	47.1
Plasma	4.527	1.242	0.004	352.5	271.4	102.0
Liver	11.740	5.073	0.024	315.2	245.9	109.0
Kidney	11.220	3.517	0.011	307.9	221.7	85.3
Testes	0.668	0.342	0.004	66.5	77.4	28.5
Skin	0.978	0.259	0.003	119.2	93.4	31.7
Carcass	0.906	0.355	0.003	97.8	103.3	33.2
	Dose-normalized concentration (mean values)					
Erythrocytes	0.951	0.220	0.002	0.212	0.156	0.045
Plasma	2.498	0.663	0.003	0.376	0.287	0.098
Liver	6.487	2.701	0.013	0.336	0.260	0.105
Kidney	6.197	1.873	0.006	0.328	0.235	0.082
Testis	0.369	0.182	0.002	0.071	0.082	0.028
Skin	0.540	0.138	0.001	0.127	0.099	0.031
Carcass	0.500	0.190	0.002	0.104	0.110	0.032
	Radioactivity as % of dose administered (mean values)					
Erythrocytes	1.42	0.34	0.003	0.28	0.23	0.06
Plasma	3.52	0.98	0.003	0.52	0.42	0.14
Liver	22.92	9.23	0.067	1.21	0.86	0.38
Kidney	4.83	1.48	0.005	0.26	0.18	0.07
Testis	0.42	0.24	0.002	0.08	0.09	0.04
Skin	12.71	3.22	0.031	2.97	2.37	0.71
Carcass	28.30	10.55	0.097	5.87	6.10	1.81

From Klempner (2006b)

good agreement with the results for rats at the lower dose in the study of absorption, distribution, metabolism and excretion.

Similar results were obtained for rats at the higher dose. Parent compound was not detected in any sample or tissue. Spirotetramat-enol was the main metabolite in all samples. Spirotetramat-desmethyl-enol was the second most prominent metabolite in the urine, plasma and organs. Significant proportions of spirotetramat-ketohydroxy were present in the liver and kidney only. Spirotetramat-enol-GA, spirotetramat-enol-alcohol and spirotetramat-desmethyl ketohydroxy were detected at low levels and were of minor importance. Identification rates were high, being in the range of 95–100%. A summary of the metabolites identified in the urine is shown in Table 9. The metabolites identified in plasma and organs of rats at the lower dose and higher dose, respectively, are summarized in Table 10 and Table 11.

Table 9. Metabolites identified in the urine of male rats given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat

Metabolite		Concentration of metabolite (% of administered dose)					
		Dose (mg/kg bw)					
		2			1000		
Sampling interval		0–1 h	0–8 h	0–24 h[a]	0–1 h	0–8 h	0–24 h[a]
1	Unknown	ND	ND	0.07	ND	ND	ND
2	Spirotetramat-enol-GA	0.04	0.40	0.55	ND	0.12	0.25
3	Unknown	ND	0.09	0.16	ND	ND	0.02
4	Unknown	ND	0.12	0.17	ND	ND	0.08
5	Unknown	ND	0.22	0.36	ND	ND	0.07
6	Unknown	ND	0.07	0.12	ND	ND	0.04
7	Unknown	ND	ND	0.07	ND	ND	0.03
8	Unknown	ND	0.10	0.13	ND	ND	ND
9	Unknown	ND	0.13	0.20	ND	ND	ND
10	Unknown	ND	ND	0.02	ND	ND	ND
11	Spirotetramat-enol-alcohol	0.26	0.81	1.15	ND	0.16	0.33
12	Spirotetramat-desmethyl-enol	1.26	20.50	33.09	0.15	4.18	10.63
13	Unknown	0.10	0.78	1.17	ND	0.16	0.29
14	Spirotetramat-desmethylketohydroxy	ND	0.13	0.20	ND	ND	0.03
16	Spirotetramat-enol	10.72	42.12	74.07	1.46	11.15	14.99
18	Spirotetramat-ketohydroxy	0.07	0.26	0.37	ND	ND	0.05
Identified		12.4	64.2	109.4	1.6	15.6	26.3
Unknown [b]		0.1	1.5	2.5	ND	0.2	0.5
Total		12.5	65.7	111.9	1.6	15.8	26.8

From Klempner (2006b)
GA, glucuronic acid; ND, not detected.
[a] Calculated sum of pooled urine from 0–4 h, 4–8 h and 8–24 h.
[b] Characterized based on their retention time in high-performance liquid chromatography (HPLC).

The first and most important metabolic reaction was the cleavage of the ester bond of the side-chain yielding the spirotetramat-enol. The demethylation of the cyclohexyl-*O*-methyl group to the respective alcohol (spirotetramat-desmethyl-enol) was a further important metabolic reaction, as well as the hydroxylation in the azaspiro ring of spirotetramat-enol, resulting in spirotetramat-ketohydroxy. Other metabolic reactions, such as conjugation of the spirotetramat-enol with glucuronic acid and oxidation of one of the methyl groups of the phenyl ring forming the spirotetramat-enol-alcohol were of minor importance.

In summary, in rats given the lower dose, absorption, distribution and excretion were rapid. Excretion was mainly renal and was nearly complete 24 h after dosing. The residues in plasma and organs declined rapidly from the maximum value at 1 h after dosing to low residues in plasma 24 h after administration. For all time-points, the residues in liver and kidney were distinctly higher than in plasma. This finding may indicate that metabolites are transferred by active transport mechanisms from plasma to the excretory organs. The residues in the testes, carcass and skin were distinctly lower than in plasma, showing the rapid excretion of the residues and the lack of accumulation of the compound. The metabolic profiles in urine were similar to those found in the study of absorption, distribution, metabolism and distribution. The ratio of the two main metabolites (spirotetramat-enol and spirotetramat-desmethyl-enol) in urine was about 2 : 1. Spirotetramat-desmethyl-enol was found at lower proportions in plasma and organs with spirotetramat-enol/spirotetramat-desmethyl-enol ratios

Table 10. Total radioactive residues of metabolites in the plasma, liver, kidney and testes of male rats given a single oral dose of [azaspirodecenyl-3-^{14}C]-spirotetramat at 2 mg/kg bw

Metabolite	Percentage of total radioactive residues[a]							
	Plasma		Liver		Kidney		Testes	
	1 h	8 h	1 h	8 h	1 h	8 h	1 h	8 h
Spirotetramat-enol-GA	ND	ND	0.1	0.2	0.4	0.4	0.1	ND
Spirotetramat-enol-alcohol	ND	ND	0.5	0.4	0.2	ND	0.2	ND
Spirotetramat-desmethyl-enol	2.3	3.6	7.3	6.8	2.3	3.6	2.5	3.3
Unknown	ND	ND	0.7	0.7	0.3	0.3	ND	ND
Spirotetramat-desmethyl-ketohydroxy	ND	ND	1.2	0.7	0.6	0.9	ND	ND
Unknown	ND	ND	0.3	0.2	0.5	0.4	ND	ND
Spirotetramat-enol	97.1	96.4	72.1	79.1	73.3	74.4	94.8	83.8
Unknown	ND	ND	1.0	0.3	1.7	1.1	ND	ND
Spirotetramat-ketohydroxy	0.6	ND	13.2	8.1	20.0	18.7	2.3	2.4
Unknown	ND	ND	0.1	ND	0.3	ND	ND	ND
Unknown	ND	ND	ND	ND	ND	ND	ND	9.4
Unknown	ND	ND	ND	ND	ND	ND	ND	0.7
Subtotal	100.0	100.0	96.5	96.5	99.5	99.7	99.9	99.4
Identified	100.0	100.0	94.4	95.3	96.8	97.9	99.9	89.4
Unknown[b]	ND	ND	2.1	1.2	2.7	1.8	ND	10.0
Not analysed/solids	—	—	3.5	3.5	0.5	0.3	0.1	0.6
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

From Klempner (2006b)

GA, glucuronic acid; ND, not detected.

[a] Values for total radioactive residues mg of equivalents of active substance/kg are presented in Table 8.

[b] Characterized on the basis of their retention time in high-performance liquid chromatography (HPLC).

Table 11. Total radioactive residues of metabolites in plasma, liver, kidney and testes of male rats given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat at 1000 mg/kg bw

Metabolite	Percentage of total radioactive residuesa											
	Plasma			Liver			Kidney			Testes		
	1 h	8 h	24 h	1 h	8 h	24 h	1 h	8 h	24 h	1 h	8 h	24h
Spirotetramat-enol-GA	ND	ND	ND	0.5	1.2	1.0	0.5	1.0	1.1	ND	ND	ND
Spirotetramat-enol-alcohol	ND	ND	ND	0.7	0.8	ND	0.4	ND	ND	ND	ND	ND
Spirotetramat-desmethyl-enol	3.3	7.5	6.8	11.6	14.7	13.0	4.9	13.1	11.5	2.6	6.4	8.7
Spirotetramat-desmethyl-ketohydroxy	ND	ND	ND	1.0	1.7	1.8	1.1	3.4	3.6	ND	ND	ND
Spirotetramat-enol	96.7	92.5	93.2	76.4	72.0	74.1	75.4	64.9	65.9	94.9	91.7	89.3
Unknown	ND	ND	ND	ND	ND	ND	1.2	1.3	1.2	ND	ND	ND
Spirotetramat-ketohydroxy	ND	ND	ND	5.5	6.8	6.6	16.3	16.0	16.2	2.2	1.4	1.1
Subtotal	100.0	100.0	100.0	95.6	97.1	96.4	99.7	99.7	99.5	99.7	99.5	99.1
Identified	100.0	100.0	100.0	95.6	97.1	96.4	98.6	98.5	98.4	99.7	99.5	99.1
Unknown [b]	ND	ND	ND	ND	ND	ND	1.2	1.3	1.2	ND	ND	ND
Not analysed/solids	—	—	—	4.4	2.9	3.6	0.3	0.3	0.5	0.3	0.5	0.9
Total:	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

From Klempner (2006b)

GA, glucuronic acid; ND, not detected.

[a] Values for total radioactive residues in mg of equivalents of active substance/kg are given in Table 8.

[b] Characterized on the basis of their retention time in high-performance liquid chromatography (HPLC).

of 10 : 1 to 40 : 1. This demonstrates that spirotetramat-desmethyl-enol was excreted more rapidly than spirotetramat-enol. The highest proportions of spirotetramat-desmethyl-enol were detected in liver, where the compound is formed by metabolic transformation of spirotetramat-enol. The proportions in plasma, kidney and testis were comparable and significantly lower than in liver. Spirotetramat-ketohydroxy was found as a prominent metabolite in the liver and kidney, but was only present at trace concentrations in the plasma, testes and urine.

In rats at the higher dose, absorption and excretion were slower and lower than at the lower dose. Only 27% of the administered dose was renally excreted 24 h after administration. The residues in the plasma were slightly higher than in the liver and kidney. This may result from the saturation of the active transport mechanisms at high concentrations, resulting in a more uniform distribution of the compound in the body. The decline of residues was slower than in rats at the lower dose. As observed in rats at the lower dose, the residues in the testes, carcass and skin were lower than in the plasma. Plasma and all organs showed slow depletion of radioactive residues. The metabolism was similar to that found in rats at the lower dose, with the exception that spirotetramat-desmethyl-enol was found at higher proportions in rats at the higher dose. As in rats at the lower dose, spirotetramat-desmethyl-enol was found at higher proportions in the urine (the ratio of spirotetramat-enol/spirotetramat-desmethyl-enol was 1.5 : 1) than in plasma and organs (ratios of 5 : 1 to 35 : 1) due to the rapid excretion of this metabolite. The highest percentages of spirotetramat-desmethyl-enol were detected in the liver as well as in kidney. The percentages in the plasma, kidney and testes were lower and comparable. Significant percentages of spirotetramat-ketohydroxy were present in the liver and kidney only.

On the basis of all the results, the Meeting concluded that due to the saturation of active transport mechanisms in the excretory organs after administration of higher doses, the depletion of residues and the excretion via the urine and faeces is slow, with a potential for accumulation in the body after repeated high doses. Moreover it can also be inferred that the pharmacokinetic parameter in rats given doses of 2 or 100 mg/kg bw are not significantly different, although they are in rats at 1000 mg/kg bw. It is possible that there is also saturation of absorption from the gastrointestinal tract in rats at at 1000 mg/kg bw. The study complied with GLP and a statement of QA was provided (Klempner, 2006b).

In a study to investigate the distribution pattern of spirotetramat and its metabolites, male (age 9 weeks) and female (age 12–13 weeks) Wistar Hsd/Cpb:WU rats were given a single dose of azaspirodecenyl-3-^{14}C-labelled spirotetramat (purity, > 98%) at a target dose of 3 mg/kg bw by oral gavage. Quantitative whole-body autoradiography (QWBA) using a radioluminography (RLG) technique allowed visualization of selective enrichments of total radioactivity, particularly for certain parts of organs or tissues that are difficult or impossible to sample during the sectioning of the animal, rendering the determination of the concentration of radiolabel in situ arduous. The data were obtained over 7 days (males) and 5 days (females) after dosing. Eight males and eight females were killed 1, 4, 8, 24, 48, 72, 120 and 168 h after dosing. The study complied with GLP and a statement of QA statement was provided.

Spirotetramat was readily absorbed from the gastrointestinal tract, distributed among almost all organs and tissues and excreted rapidly within approximately 24 h (females) or 48 h (males). Excretion was mainly renal. Only a minor amount (approximately 3–6%) was excreted via the faeces. No radiolabel was detected in the expired air, showing the stability of the labelling position in the molecule. QWBA revealed fast absorption and distribution of the test compound with peak values observed within 1 h after dosing. Among the organs, tissues and fluids analysed quantitatively, the highest concentrations of equivalents were observed in the liver, kidney and blood. Moderate peak concentrations were found in the lungs and myocardium, brown fat, skin, the glands and the reproductive organs. Lower concentrations were found in all other organs and tissues. The lowest peak

concentrations were found in the spinal cord, the brain and the eye. From peak values, concentrations of radiolabel declined by several orders of magnitude to below the limit of detection for all organs and tissues within 48 h in males and within 24 h in females. Only in the liver of males were very low residues (slightly above the limit of quantitation) detected at 72 h after dosing. Based on these results, the distribution of radiolabel and corresponding metabolites in the organs and tissues of male and female rats is considered to be sufficiently understood. The TRR values for the organs and tissues in male and female rats, respectively, are shown in Tables 12 and 13 (Klempner, 2006c).

1.2 Metabolism in vitro

A comparative study of metabolism in vitro in male rats, mice and humans revealed differences in the proportions of metabolites formed by the different species. This study, which complied with GLP and for which a QA statement was provided, used Liverbeads™ –immobilized hepatocytes entrapped within an alginate matrix. The study was carried out with spirotetramat at two concentrations

Table 12. Distribution of radiolabel in organs and tissues of male rats given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat at 3 mg/kg bw

Organ/tissue	Total radioactive residues (µg active substance equivalents/g wet weight)							
	Time of sacrifice (h)							
	1	4	8	24	48	72	120	168
Blood	2.711	1.285	0.277	< LOQ	—	—	—	—
Liver	7.437	5.439	1.173	0.017	< LOQ	0.006	< LOQ	< LOD
Renal cortex	10.635	4.811	1.429	0.008	< LOD	—	—	—
Renal medulla	12.723	7.614	2.391	0.008	< LOD	—	—	—
Brown fat	1.247	0.626	0.177	—	—	—	—	—
Perirenal fat	—	0.076	0.043	< LOD	—	—	—	—
Skeletal muscle	0.658	0.331	0.064	—	—	—	—	—
Myocardium	1.611	0.738	0.177	< LOD	—	—	—	—
Lung	1.097	0.501	0.196	< LOD	—	—	—	—
Spleen	0.537	0.255	0.065	< LOD	—	—	—	—
Pancreas	0.591	0.306	0.068	< LOD	—	—	—	—
Bone marrow	0.681	0.298	0.088	—	—	—	—	—
Testes	0.509	0.404	0.088	< LOD	—	—	—	—
Brain	0.102	0.056	0.012	< LOD	—	—	—	—
Spinal cord	0.106	0.058	0.011	—	—	—	—	—
Pitutary gland	0.966	0.599	0.101	—	—	—	—	—
Pineal body	1.023	0.479	0.106	—	—	—	—	—
Adrenal gland	2.005	0.683	0.168	—	—	—	—	—
Thymus	0.579	0.236	0.053	< LOD	—	—	—	—
Thyroid gland	1.412	0.639	0.142	—	—	—	—	—
Salivary gland	1.222	0.506	0.100	—	—	—	—	—
Nasal mucosa	0.439	0.319	0.061	—	—	—	—	—
Skin	0.955	0.572	0.106	—	—	—	—	—
Vitreous body (eye)	0.146	0.037	0.024	—	—	—	—	—

From Klempner (2006c)
< LOD, less than limit of detection; < LOQ, less than limit of quantitation.

i.e. 50 µmol/l and 520 µmol/l. At the lower concentration, analysis of the Liverbeads™ samples using liquid chromatography/mass spectrometry (LC/MS) revealed five different metabolites of spirotetramat in mice, four in rats and three in humans. Spirotetramat itself was not detected in the Liverbeads™ from any species tested. The major metabolites detected in the mouse were spirotetramat-enol and spirotetramat-enol-GA, occurring at 66% and 30%, respectively. The glucurono-conjugation of the main metabolite spirotetramat-enol appeared to be a major route in the in-vitro degradation (detoxification) of spirotetramat in the mouse. Spirotetramat-enol-alcohol, spirotetramat-desmethyl-enol and spirotetramat-ketohydroxy were detected at a very low level in the mouse (1–2%). In the rat, the main metabolites were spirotetramat-enol and spirotetramat-desmethyl-enol, occurring at 87% and 7% respectively. Spirotetramat-enol-alcohol and spirotetramat-ketohydroxy represented 4% and 3% respectively. The spirotetramat-enol-GA (enol-glucuronide) was not detected in vitro in the rat. The major metabolites detected in humans were spirotetramat-enol and spirotetramat-enol-GA

Table 13. Distribution of radiolabel in organs and tissues of male rats given a single oral dose of [azaspirodecenyl-3-^{14}C]spirotetramat at 3 mg/kg bw

Organ/tissue	Total radioactive residues (µg active substance equivalents/g wet weight)						
	Time of sacrifice (h)						
	1	4	8	24	48	72	120
Blood	1.195	0.365	0.088	—	—	—	—
Liver	4.497	1.318	0.397	< LOD	—	—	—
Renal cortex	5.148	1.489	0.438	—	—	—	—
Renal medulla	7.306	2.624	0.913	—	—	--	—
Brown fat	0.530	0.116	0.037	—	—	—	—
Perirenal fat	0.108	0.053	0.010	—	—	—	—
Skeletal muscle	0.247	0.070	0.018	—	—	—	—
Myocardium	0.749	0.198	0.052	—	—	—	—
Lung	0.823	0.116	0.043	—	—	—	—
Spleen	0.241	0.068	0.017	—	—	—	—
Pancreas	0.281	0.074	0.021	—	—	—	—
Bone marrow	0.268	0.089	0.020	--	—	—	—
Ovary	0.595	0.111	0.028	—	—	—	—
Uterus	0.759	0.170	0.045	—	—	—	—
Brain	0.047	0.013	< LOQ	—	—	—	—
Spinal cord	0.051	0.014	< LOQ	—	—	—	—
Pitutary gland	0.476	0.134	0.032	—	—	—	—
Pineal body	0.504	0.122	0.038	—	—	—	—
Adrenal gland	0.877	0.216	0.065	—	—	—	—
Thymus	0.214	0.066	0.015	—	—	—	—
Thyroid gland	0.567	0.162	0.036	—	—	—	—
Salivary gland	0.578	0.147	0.031	—	—	—	—
Nasal mucosa	0.172	0.074	0.014	—	—	—	—
Skin	0.564	0.140	0.032	—	—	—	—
Vitreous body (eye)	0.060	0.040	0.009	—	—	—	—

From Klempner (2006c)
< LOD, less than limit of detection; < LOQ, less than limit of quantitation.

(enol-glucuronide), but their relative abundance was different from that observed in the mouse, occurring at 92% and 6%, respectively. Spirotetramat-desmethyl-enol was present at a very low level (1%); spirotetramat-enol-alcohol and spirotetramat-ketohydroxy were not detected in humans.

At the higher concentration, no additional metabolites were detected beyond those seen in low concentrations in the rat, mouse and human Liverbeads™. The low degree of metabolism observed in this experiment could be indicative of a saturation of the biotransformation enzymatic system.

At the lower concentration, spirotetramat was thus completely metabolized in the liver cells from all species and no parent compound was detected at the end of the incubation. Spirotetramat-enol was the first and most prominent metabolite, accounting for 66–92% of total metabolites. The metabolic profile among the three species at low concentration showed marked differences. In the rat, the spirotetramat-enol was further metabolized by oxidation reactions to spirotetramat-desmethyl-enol (oxidative demethylation), spirotetramat-enol-alcohol (oxidation of aromatic methyl group) and spirotetramat-ketohydroxy (oxidation of the azaspirodecenyl moiety). Oxidation products accounted for about 14%. Conjugation was not detected as an in-vitro metabolic transformation. The general picture of the in-vitro metabolic pathway in the rat was very similar to in-vivo metabolism in the male rat. The same metabolites were detected with spirotetramat-enol and spirotetramat-desmethyl-enol as the two most important degradation products, although the proportions of metabolites were different in vivo and in vitro. In the mouse, oxidative degradation of spirotetramat-enol was detected as a minor metabolic reaction in vitro only (4% of oxidation products). Conjugation to spirotetramat-enol-GA was very prominent with the conjugate accounting for about 30%. Human liver cells showed an in-vitro metabolism that was more similar to that found in the mouse than in the rat. Conjugation to spirotetramat-enol-GA (6%) was more prominent than oxidative transformation, which was only detected as a minor transformation (1%). The results of this study are summarized in Table 14 (Totis, 2006).

1.3 Modelling of physiologically-based pharmacokinetics

Physiologically-based pharmacokinetic (PBPK) simulations were carried out with the commercially available software PK-Sim (Bayer Technology Services GmbH). PK-Sim is based on a generic whole-body PBPK model which describes the uptake and distribution of organic substances

Table 14. Relative distribution of the metabolites in hepatocytes of mice, rats and humans

Species	Metabolite (percentage of administered dose)					
	Spirotetramat-enol-glucuronide (M = 477)	Spirotetramat-enol alcohol (M = 317)	Spirotetramat-desmethyl-enol (M = 287)	Spirotetramat-enol (M = 301)	Spirotetramat-keto hydroxy (M = 317)	Spirotetramat (M = 373.45)
Spirotetramat, 50 µmol/l						
Rt (min)	15.2–15.4	25.4–25.8	35.5–36.4	45.6–45.8	47.1	59.2
Rat	0	4	7	87	3	0
Mouse	30	1	1	66	2	0
Human	6	0	1	92	0	0
Spirotetramat, 520 µmol/l						
Rat	0	0	0	100	0	0
Mouse	9	1	0	89	1	0
Human	2	0	0	98	0	0

From Totis (2006)
M, mass; Rt, retention time.

after oral or intravenous administration. The experimental data used in the model are shown in Table 15.

The model showed that the behaviour of spirotetramat in male rats is well described by physiology-based simulations. It is possible to find a single parameterization of the PBPK model in such a way that observed plasma concentrations, their nonlinearity in dose, organ concentrations and metabolism and excretion can be described by the simulation with excellent agreement with experimental data. Strongly increased concentrations of spirotetramat in the liver and kidney compared with the plasma and other tissues, as observed by QWBA (quantitative whole-body autoradiography), indicate the presence of active-transport processes for the uptake of spirotetramat metabolites into these tissues. Moreover, the renal excretion rate of spirotetramat-enol can only be explained by active tubular secretion into the urine, because the physicochemical properties yield a glomerular filtration rate that is much smaller than the observed rate of excretion. It was shown that saturation of the renal transport processes is highly likely to be responsible for the experimentally observed nonlinearity of plasma concentrations of spirotetramat at doses of 2 mg/kg bw and 100 mg/kg bw. While the available experimental data do not indicate which of the two processes, uptake or excretion, is most critical, it is reasonable to assume that in reality there is simultaneous saturation of both processes, as unbound concentrations comparable to typical binding constants (in the pmol range) for transport proteins are reached or exceeded in the plasma as well as the kidney cells.

The PBPK simulations allowed extrapolation to doses higher than those investigated experimentally in studies of sbsorption, distribution, metabolism and excretion. Calculations were carried out for doses up to 1000 mg/kg bw, covering the range that has been studied in toxicological experiments. It was found that the saturation of the active-transport processes responsible for transferring metabolites from plasma to excretory organs leads to a strong change in the shape of the plasma concentration curve at the highest doses. This has in turn a significant impact on pharmacokinetic parameters, describing the systemic exposure to a compound. While the dose-normalized maximum concentrations show only a immoderate increase with dose, this increase is rather high for the area under the concentration curve. The AUC_{norm} is about five times higher at 1000 mg/kg bw than at 2 mg/kg bw. This means that the increase in overall systemic exposure is five times higher than expected in the usually assumed case of dose linearity. An even more pronounced change was found for the peak/trough ratio $[C_{max}/C_{(24h)}]$. This parameter decreases by a factor of approximately 500, reaching values as low as 5–6 at 1000 mg/kg bw. Such low peak/trough ratios are indicative of a potential continuous rise of systemic concentrations upon repeated daily administration. In fact, such a rise in body burden was found for doses above 500 mg/kg bw in simulations of daily administration for

Table 15. Experimental data used for a physiologically-based pharmacokinetic model with spirotetramat

Title	Data used	Reference
[Azaspirodecenyl-3-14C]-spirotetramat: absorption, distribution, excretion and metabolism in the rat	Plasma concentrations (total radioactivity) at doses of 2 mg/kg bw and 100 mg/kg bw.	Klempner (2006b)
	Metabolite spectrum in excreta at doses of 2 mg/kg bw and 100 mg/kg bw	
[Azaspirodecenyl-3-14C]spirotetramat: distribution of the total radioactivity in male and female rats determined by quantitative whole-body autoradiography (QWBA) including determination of the total radioactivity in excreta and exhaled 14CO2	Tissue concentrations (total radioactivity) in peripheral organs	Klempner (2006a)
[Azaspirodecenyl-3-14C]-spirotetramat: comparison of the in vitro metabolism in Liverbeads™ from rat, mouse and human.	Fraction of desmethyl-enol detected after incubation with spirotetramat at 50 µmol/l for 4 h	Totis (2006)

From Schmitt (2006a)

4 weeks. At 1000 mg/kg bw, the mean daily concentrations increased about twofold with time until a steady state was reached after about 75 days. This led to an even more marked non-linearity of the AUC, with a sevenfold increase of AUC_{norm} between 2 mg/kg bw and 1000 mg/kg bw, compared with fivefold after a single dose.

A sensitivity analysis also revealed that under certain circumstances peak/trough ratios considerably less than 5 will be obtained. This is the case if either the fraction of dose absorbed from the gut is higher or the metabolic rate for the transition of enol to desmethyl-enol is lower at high doses than in the present simulations. The actual values of the properties might generally differ from those of the simulation because they rely on parameter values estimated with some uncertainty. An assessment of the dependence of the disproportional increase in systemic exposure on the peak/trough ratio showed that a distinct change in behaviour occurs between ratios of 5 and 3. Below that range, the moderate temporal increase of systemic exposure described above changes into a strong one, with concentrations increasing continuously over the 4 weeks investigated here. This leads to systemic exposures (AUC) to the active substance at doses that are about 20 times higher than those extrapolated linearly from values at the lowest dose. Although this case was not predicted here even for the highest dose of 1000 mg/kg bw, it cannot be excluded that it actually occurs. A sensitivity analysis revealed that within the uncertainty range of estimated model parameters, peak/trough ratios that are even lower than 3 could be obtained. This is the case if the binding constant of spirotetramat to the hepatic-uptake transport protein is actually lower than estimated here, a case that is reasonable, but needs further experimental data to be confirmed or rejected. The disproportionate increase in plasma concentration after repeated administration of spirotetramat might have an impact on systemic exposure after high doses in toxicological studies. However without further supporting data, the simulations at doses greater than 100 mg/kg bw should be viewed with caution, since the PBPK model was developed using experimental data only up to a dose of 100 mg/kg bw (Schmitt, 2006a).

A more refined PBPK model using the results of the study of absorption, distribution, metabolism and excretion (Klempner, 2006a) and the organ metabolism study (Klempner, 2006b) predicted a distinct, disproportionate increase of the body burden by spirotetramat-enol and spirotetramat-desmethyl-enol after repeated administration of very high doses of spirotetramat due to saturation of active transport process for this substance. Using the most recent experimental information about tissue concentrations in liver and kidney at the highest dose and the concentrations of the metabolite desmethyl-enol, parameterization of the PBPK-model for spirotetramat could be improved significantly. The results of the PBPK-simulations show a good agreement with observed data for the time-course of the plasma and tissue concentrations of spirotetramat-enol and spirotetramat-desmethyl-enol as well as for the excreted amounts. In most cases the calculated data show deviations from those observed within or below a factor of two. For the dose of 1000 mg/kg, calculated enol concentrations were four–seven-fold higher than those determined experimentally. No explanation for this discrepancy could be found, although several hypotheses were tested. However, other results that depend on the spirotetramat-enol plasma concentration (e.g. the excreted amounts of spirotetramat-enol and spirotetramat-desmethyl-enol or the spirotetramat-desmethyl-enol plasma and tissue concentrations) are in good agreement with the experimental data. Moreover, it could be shown that the saturation of the transport processes involved in the excretion is predominantly responsible for the decreases in peak/trough ratio at high doses that were experimentally confirmed for 1000 mg/kg bw.

For the application of the model in simulations of scenarios not investigated experimentally it is, however, important that, despite the discrepancy in absolute concentrations found at high doses, the general pharmacokinetic behaviour with the strong reduction of $C_{max}/C_{(24h)}$ with increasing dose is well represented by the calculations. The reduction of the peak/trough ratio is the main reason for the strong rise of concentrations predicted for repeated administration of doses larger than 300 mg/kg bw. While for the dose of 100 mg/kg bw per day no long-term increase in plasma concentration

was observed, this is clearly the case at 500 mg/kg bw per day. At 300 mg/kg bw, there is only a slight increase of about a factor of two over 4 weeks. Simulations of repeated daily administration of spirotetramat for 4 weeks revealed that mean daily plasma concentrations of spirotetramat-enol remained constant over time after doses of 10 or 100 mg/kg bw per day, but rising plasma concentrations were seen after dosing with 300 mg/kg bw per day due to the beginning of saturation of the uptake process into liver and kidney cells. It is predicted that mean daily plasma concentrations of spirotetramat-desmethyl-enol also remain constant after doses of 10 or 100 mg/kg bw per day, increase linearly with time at daily doses of 300 or 500 mg/kg bw per day and increase nearly 100-fold after daily administration of 1000 mg/kg bw per day. As was hypothesized by the authors of the previously described PBPK-modelling study the disproportionate increase in plasma concentration of the metabolites after repeated administration of spirotetramat might have an impact on systemic exposure after high doses in toxicological studies (Schmitt, 2006b).

2. Toxicological studies

2.1 Acute toxicity

(a) Oral toxicity

In a study of acute oral toxicity, groups of five fasted female Wistar rats were given spirotetramat (purity, 93.5%) at a dose of 2000 mg/kg bw by gavage. All the rats survived and gained weight during the study. No clinical signs of toxicity were observed, nor were there any findings at necropsy. The median lethal dose (LD_{50}) was > 2000 mg/kg bw (Eigenberg, 2004a).

(b) Dermal toxicity

In a study of dermal toxicity, groups of five male and five female Wistar rats were given spirotetramat (purity, 93.5%) at a dose of 2000 mg/kg bw applied dermally. All the rats survived and gained weight during the study. Clinical signs observed on days 0–3 were: red stained-nose, wetness-urogenital area, yellow-stained urogenital area, and red-coloured skin on the back. There were no findings on necropsy. The dermal LD_{50} for male and female rats was > 2000 mg/kg bw (Eigenberg, 2004b).

(c) Exposure by inhalation

In a study of exposure by inhalation, groups of five male and five female Wistar rats were exposed to spirotetramat (purity, 96.5%) at mean solid aerosol concentrations of 1.10 or 4.18 mg/l air by nose-only exposure for 4 h followed by a 2-week observation period. Attempts were made to make the aerosols generated respirable to rats. With regard to the respirability of the aerosol generated, internationally recognized recommendations such as SOT (Society of Toxicology, 1992) were fulfilled, i.e. the median mass aerodynamic diameter (MMAD) was 3.7–5.1 μm (geometric standard devaition, GSD 2.3).

Exposure to spirotetramat at concentrations of up to 4.18 mg/l air did not result in mortality. The following clinical signs were observed: ungroomed hair-coat, piloerection, bradypnoea, laboured breathing, dyspnoea, breathing sounds, **reddened** nostrils, nasal discharge (serous), **red** nostrils **with** encrustations, **red encrustations in** nose/snout region:, stridor, reduced motility, limpness, high-legged gait, impaired reflexes, hypothermia, and decreased body weights. The duration of signs was governed by respiratory effects indicative of irritation of the lower and upper respiratory tract and resolved towards the beginning of the second week after exposure. Findings on necropsy were unremarkable. The median lethal concentration (LC_{50}) of spirotetramat was > 4.18 mg/l air (Pauluhn, 2002).

(d) Dermal irritation

In a study of acute dermal irritation, three male rabbits were given 500 mg of spirotetramat (purity, 96.5%) applied dermally under a patch to the shaved intact dorsal skin. After a 4-h exposure period, the patch was removed and the skin sites were evaluated. Scores were taken 60 min, 24 h, 48 h and 72 h after patch removal. None of the three rabbits showed any test substance-related lesions. There were no systemic intolerance reactions. Spirotetramat is not a skin irritant (Leuschner, 2002a).

(e) Ocular irritation

To examine the effect of spirotetramat on rabbit eyes, three male rabbits were each given a single application of 100 mg of spirotetramat (purity, 96.5%) into the conjunctival sac of the right eye. Corneal opacity (grade1) was observed in all rabbits 24 h to 6 days after instillation and in two rabbits up to 7 days after instillation. Irritation of the iris (grade 1) was observed in all rabbits (rabbit No. 1, 24 h to 6 days after instillation; rabbit No. 2, 72 h to 5 days after instillation; and rabbit No. 3, 24 h to 5 days after instillation). Conjunctival redness (grade 1) was observed in all rabbits 1 h to 72 h after instillation and in Nos 2 and 3 until 4 days after instillation. Conjunctival chemosis (grade 1) was noted in rabbit No. 3 24 h and 48 h after instillation. There were no systemic intolerance reactions. Spirotetramat is an eye irritant (Leuschner, 2002b).

(f) Dermal sensitization

Guinea-pigs

In a maximization test that complied with OECD guideline No. 406, EC guideline 96/54/EC (22nd adaptation of guideline 67/548/EEC) and health effects test guideline OPPTS 870.2600, 20 female guinea-pigs were given spirotetramat (purity, 96.5%; formulated in polyethylene glycol 400 to yield a suspension) at the following concentrations: intradermal induction, 5%; topical induction, 50%; challenge, 25%. Two additional guinea-pigs were used for dose-finding and the control group comprised 10 guinea-pigs. Challenge with spirotetramat at 25% produced skin effects (grade 1–3) in 18 out of 19 guinea-pigs (95%). No skin effects were seen in guinea-pigs in the control group. Under the conditions of the maximization test and with respect to the evaluation criteria, spirotetramat exhibits a skin-sensitization potential (Vohr, 2002).

In a Buehler epicutaneous patch test that complied with OECD guideline No.406, EC guideline 96/54/EC (22nd adaptation of guideline 67/548/EEC and health effects test guideline OPPTS 870.2600, 20 female guinea-pigs were given spirotetramat (purity, 97.2%; formulated in polyethylene glycol 400 to yield a suspension or a paste) at the following concentrations: first to third induction**:**, 71%; challenge**:**71%. The control group comprised 10 guinea-pigs. Two additional guinea-pigs were used for dose-finding for the challenge concentration. There were no skin effects after induction or challenge in either the control group or the group receiving spirotetramat. The Meeting concluded that spirotetramat has no skin-sensitization potential (Vohr, 2004).

Mice

The local lymph-node assay (LLNA) was used to assess dermal contact sensitization in mice treated with spirotetramat. Groups of five female CBA/J mice were treated with spirotetramat (purity, 97.2%) at a concentration of 1%, 2.5%, 5% or 10%. Additional groups were treated with isoeugenol, the positive control, at a concentration of 0.5%, 1%, 2.5% or 5%. One additional group received dimethylformamide, the vehicle, only. The test substances were applied on the external surfaces of each

ear (i.e. 50 µl per mouse) for three consecutive days (days 0, 1 and 2) at the appropriate concentration. On day 5, the cell proliferation in the local lymph nodes was measured by incorporation of tritiated methyl-thymidine and the values obtained were used to calculate proliferation indices.

No mortality and no clinical signs of toxicity were observed during the study. No cutaneous reactions were observed in any group. The proliferation indices for spirotetramat were 3.4, 4.3, 5.4 and 5.9 at concentrations of 1%, 2.5%, 5% and 10%, respectively. The proliferation indices for the positive control were 0.8, 1.3, 1.8 and 3.4 at concentrations of 0.5%, 1%, 2.5% and 5%, respectively. The skin sensitization potential of spirotetramat was approximately five times that of isoeugenol, the positive control (Esdaile, 2004).

2.2 Short-term studies of toxicity

Mice

In a 4-week dose range-finding study that did not comply with GLP, groups of five male CD-1 mice were fed diets containing spirotetramat (purity, 97%) at a concentration of 0, 500 or 5000 ppm, equivalent to 0, 136.5 and 1415 mg/kg bw per day. The method of calculating compound intake (mg/kg bw per day) was not reported

There was no mortality or any treatment-related clinical findings, apart from diminished food intake compared with the control group (500 ppm, –19%; 5000 ppm, –16%) and no other toxicological findings were noted.

Table 16. Acute toxicity of spirotetramat

Species	Strain	Sex	Route	LD50 (mg/kg bw)	LC50 (mg/l air)	Other effects	Reference
Rat	Wistar	Female	Oral	> 2000	—	—	Eigenberg (2004a)
Rat	Wistar	Male and Female	Dermal	> 2000	—	—	Eigenberg (2004b)
Rat	Wistar	Male and female	Inhalation (4-h, nose only) (MMAD was 3.7–5.1 pm, solid aerosol)*	—	> 4.18	—	Pauluhn (2002)
Rabbit	Himalayan	Male	Dermal irritationa	—	—	Not an irritant	Leuschner (2002a)
Rabbit	Himalayan	Male	Ocular irritationa	—	—	Irritant	Leuschner (2002b)
Guinea-pig	SPF strain Hsd Poc:DH	Female	Dermal sensitization (maximization)[a]	—	—	Exhibits a skin-sensitization potential	Vohr (2002)
Guinea-pig	SPF strain Crl:HA	Female	Skin sensitization (Buehler patch test)[a]	—	—	No evidence of skin sensitization	Vohr (2004)
Mice	CBA/J	Female	Skin sensitization effects (local lymph node assay)[a]	—	—	Skin-sensitizing potential	Esdaile (2004)

[a] The test material used for inhalation, eye and primary irritation and other sensitization studies is a *cis*-isomer of the TGAI test material used for the acute oral and acute dermal studies.
MMAD, mass median aerodynamic diameter.

The no-observed-adverse-effect level (NOAEL) was 5000 ppm, equivalent to 1415.2 mg/kg bw per day, on the basis of pathology and the organ investigated **(liver/gall bladder, adrenal glands, testes and epididymides)** (Schladt, 2001).

In a study that complied with GLP and for which a statement of quality assurance (QA) was provided, groups of 15 male and 15 female CD-1(ICR)/BR mice were given diets containing spirotetramat (purity, 93.1–96.5%) at nominal dietary concentrations of 0, 70, 350, 1700 or 7000 ppm, equal to 0, 13, 60, 300 and 1305 mg/kg bw per day for males and 0, 6, 72, 389 and 1515 mg/kg bw per day for females, for approximately 14 weeks. The mice were aged approximately 10 weeks at the initiation of the study. The objectives of this study were to obtain a toxicological profile for spirotetramat under conditions of prolonged and repeated exposure in the mouse, to establish doses for a subsequent lifetime exposure study and also to design a study to assess the oncogenic potential in this animal.

After approximately 14 weeks of continuous dietary exposure to the test substance, no toxicologically relevant response was observed.

The NOAEL was 7000 ppm, equal to1305 mg/kg bw per day, on the basis of lack of findings at any dose tested (Wahle, 2005a).

Rats

In a study of dermal administration, which complied with GLP and for which a statement of QA was provided, groups of 10 male and 10 female Wistar Hanover CRL:WI(GLX/BRL/HAN)IGS BR rats were given spirotetramat (purity, 97.6–98.5%) dermally at a dose of 0, 100, 300 or 1000 mg/kg per day for 28 or 29 days. The rats were aged approximately 8–10 weeks at the initiation of study. The test substance was held in contact with the skin for a minimum of 6 h per day for five consecutive days per week for 4 weeks. The bandage and tape were removed each day and the application site was gently wiped with water-dampened gauze and then with dry gauze to remove as much test substance residue as feasible without damaging the skin. The resulting mean dose was approximately 700 mg/kg bw per day at the highest dose. During the dosing period, clinical observations were conducted daily and body weights were measured weekly. Ophthalmic examinations were performed once before administration of the test substance and during week 4 of the study. Clinical chemistry and haematology analyses were performed on all rats during week 3 of the study, except for prothrombin time and thromboplastin time which were analysed during week 4. A gross necropsy was performed, organ weights were taken and tissues were examined microscopically.

Since no treatment-related effects were observed in males or females at any dose, the NOAEL was 1000 mg/kg bw per day, the highest dose (Eigenberg, 2006a).

In a 4-week study that did not comply with GLP, groups of five female Hsd/Win:WU rats were diets containing spirotetramat (purity, 98.2%) at a concentration of **0**, 500 or 5000 ppm (equal to **0**, 47.3 and 501.8 mg/kg per day) for 4 weeks.

The intake of test compound was proportional to the dietary concentration.

No clinical signs of toxicity were observed and survival was unaffected by treatment with spirotetramat. Values for body weight, body-weight gain and food intake of treated rats were comparable to those for rats in the control group. Clinical chemistry examination revealed a non-dose-related decrease in triglyceride concentrations in rats at 500 and 5000 ppm.The liver weight of treated rats was comparable to that of rats in the control group. Gross pathology and histopathology showed no treatment-related effects. The liver-cell proliferation assay showed no relevant increase in cell proliferation or in nuclear-area values for treated rats. The study author concluded that a 4-week treatment with spirotetramat slightly influenced lipid metabolism in female rats. However,

this finding was **not** considered to be toxicologically. The results of the present study were also supported by the findings in CD-1 mice in which dietary exposure to spirotetramat resulted in no treatment-related effects in male mice up to a dietary concentration of 5000 ppm (Krotlinger et al., 1998).

In a short-term feeding study that complied with GLP and for which a statement of QA was provided, groups of male and female Wistar Hanover rats (Crl:WI[Glx/BRL/Han]IGS BR; aged approximately 10 weeks) were given diets containing spirotetramat (purity, 93.1–96.5%) at nominal dietary concentrations of 0, 150, 600, 2500 or 10 000 ppm for approximately 14 weeks. The mean daily intake of spirotetramat over approximately 14 weeks at nominal dietary concentrations of 150, 600, 2500 or 10 000 ppm was equal to 9, 36, 148 and 616 mg/kg bw per day for males and 11, 46, 188 and 752 mg/kg bw per day for females, respectively. The control group and group at the highest dose contained 20 males and 20 females, while all other groups contained 10 females and 10 females. To assess the reversibility of any effects observed during the exposure period, 10 males and 10 females from the control group and the group at the highest dose were placed on control diet for the remainder of the study (approximately 4 weeks). The rats were aged approximately 10 weeks at the initiation of study.

Body weight and food consumption determinations as well as a detailed clinical examination of each rat were conducted weekly throughout the study. Observations for moribundity and mortality were performed once daily. Standard haematological, clinical chemistry and urine-analysis endpoints were evaluated from blood drawn via the orbital sinus (while under light anaesthesia with IsoFlo®; isoflurane); and urine collected just before the respective termination of both the exposure and recovery periods. Selected hepatic-enzyme activities were also measured. Ophthalmological examinations were conducted on all acclimatized rats before exposure, and then again on all surviving rats immediately before the respective termination of both the exposure and recovery periods of the study. All rats were examined post mortem.

At 10 000 ppm, a decrease in the rate of body-weight gain of 17.6% was noted in males only; a corresponding decrease of 8.4% was noted in absolute body weight after 14 weeks. Organ-weight changes were limited to a slight decrease in absolute testicular weight. Histopathological considerations included an increased incidence of minimal to severe abnormal spermatozoa and hypospermia in the epididymis and minimal to moderate tubular degeneration in the testis. The average incidence and severity (in parentheses) of treatment-related microscopic findings in testes and epididymis is shown in Table 17.

Table 17. Average incidence and severitya of treatment-related microscopic findings in testes and epididymis of rats given diets containing spirotetrmat for 14 weeks

Finding	Dietary concentration (ppm)						
	0	150	600	2500	10 000	0	10 000
						Recovery	Recovery
Epididymis, abnormal spermatozoa	0	—	0	0	9* (1.8)	0	1 (1.0)
Epididymis, hypospermia	0	—	0	0	5* (2.6)	0	1 (5.0)
Testes, tubular degeneration	0	—	0	0	5* (2.0)	0	1 (2.0)
Testes, vacuolization	0	—	0	0	5* (1.6)	0	0

From Wahle (2005a)

***** Significantly different from controls, $p < 0.05$.

[a] Figures in parentheses indicate average severity, graded from 1 (minimal) to 5 (severe).

An increased incidence of minimal to slight accumulation of alveolar macrophages in the lungs (males, 2, 4, 0, 5, 9; females, 1, 0, 0, 1, 7) was noted in both sexes and was statistically significant at the highest dose. The effects on all parameters showed a degree of reversibility by the end of the recovery period and many parameters had fully reverted to control values. The effects on testes and epididymis (including sperm) were reversible in most animals after cessation of treatment. No effects attributable to exposure to spirotetramat at 150, 600 and 2500 ppm were observed.

The lowest-observed-adverse-effect level (LOAEL) was 10 000 ppm, equal to 616 mg/kg bw per day, on the basis of decrease in body-weight gain in males, structural changes in the testes, and structural changes in the lungs of males and females. The NOAEL was 2500 ppm, equal to 148 mg/kg bw per day, on the basis of decreased body-weight gain in males, structural changes in the testes, abnormal spermatozoa and hypospermia and tubular degeneration and structural changes in the lungs (males and females) at 10 000 ppm, equal to 616 mg/kg bw per day (Wahle, 2005a).

In a 1-year study of oral toxicity, groups of 25 male and 25 female Wistar Hanover (Crl:WI[Glx/BRL/Han]IGS BR) rats (age approximately 9–10 weeks) were given diets containing spirotetramat (purity, 97.5–98.5%) at nominal dietary concentrations of 0, 250, 3500 or 7500 ppm (males)/12 000 ppm (females) for approximately 1 year. The mean daily intake of the test substance (mg spirotetramat/kg bw per day) over approximately 1 year at nominal dietary concentrations of 250 ppm, 3500 ppm or 7500ppm (males)/12 000 ppm (females) respectively, was equal to 13, 189 and 414 for males and 18, 255 and 890 for females. All test diets (including control) were available for consumption ad libitum at all times. The concentration of spirotetramat in the diet, as well as the homogeneity and stability of spirotetramat as a dietary admixture was confirmed.

Body weight and food consumption determinations were conducted weekly for 13 weeks and once per month (at either 4 or 5 weeks, depending on the number of days in the month) thereafter; detailed clinical examinations, including general open-field observations, of each rat were conducted weekly throughout the study. Observations for moribundity and mortality were performed at least once daily. During month 12, the last 10 rats of each sex per dose were subjected to a functional observational battery (FOB) test to assess motor activity, grip strength and sensory reactivity to stimuli of different types (e.g. visual, auditory and proprioceptive stimuli). Standard haematological, clinical chemistry and urine-analysis end-points were evaluated from blood (fasted; drawn via the orbital sinus while under light anesthesia with IsoFlo®; isoflurane) and urine collected at approximately 3, 6, and 12 months during the study. Ophthalmological examinations were conducted on all acclimatized rats before exposure, and then again on all surviving rats just before termination. All rats were given a post-mortem examination, which included documenting and saving all gross lesions, weighing designated organs, and collecting representative tissue specimens for histopathological evaluation.

At a dietary concentration of 250 ppm, no effects attributable to exposure to spirotetramat were observed.

At 3500 ppm there was an increase in the incidence of minimal to slight accumulation of alveolar macrophages in the lungs in males only. No other exposure-related effects were observed in this group.

At the highest dietary concentration, 7500 ppm for males and 12 000 ppm for females, a decline in body weight of 6.6% was noted in females, based on final live body weight (compared with controls). However male body weight was unaffected. Clinical observations included an increased incidence of yellow and brown staining in females, generally located in the perigenital area and tail. Gross observations consisted of an increased incidence of discoloration of the lung in females. Slightly increased liver weight was found in both sexes. Histopathological considerations included a statistically significantly increased incidence of minimal to slight accumulation of alveolar

macrophages in the lungs in both sexes. An increased incidence of exfoliated germ cells/debris was observed in the epididymis of males at 7500 ppm (3 out of 25 vs 0 out of 24 in controls). Abnormal spermatozoa were also observed in males at the highest dose (2 out of 25 vs 0 out of 25 in controls). Although the increases were not statitistically significant, they were considered to be toxicologically relevant. Microscopic findings are summarized in Table 18.

The NOAEL was 250 ppm, equal to 13.2 mg/kg bw per day, on the basis of an increased in the incidence of accumulation of alveolar macrophages in the lungs of males at 3500 ppm. The studies complied with GLP and a statement of QA was provided (Wahle, 2005b).

Dogs

In a 4-week dose range-finding study that complied with GLP and for which a statement of QA was provided, groups of two male and two female beagle dogs (aged 5–6 months) were given diets containing spirotetramat (purity, 93.5–96.5%) at a concentration of 0, 100, 400, 1600 or 6400 ppm, equal to 0, 3, 13, 42 and 104 mg/kg bw per day for males and 0, 3, 12, 70 and 127 mg/kg bw per day for females. Clinical observations and food consumption were measured daily, while body weights were measured weekly. Ophthalmological, neurological and heart and liver examinations were performed before exposure and before sacrifice. Clinical chemistry and haematological parameters were measured before exposure and on day 7 and on day 23, while urine analysis was conducted before exposure, then on day 9 and 24. At study termination, liver UDP-GT activity was measured, and gross necropsy, organ weight and histopathology were peformed.

In the group at 6400 ppm, the highest dose, body weight and food consumption were decreased. There were compound-related clinical findings at this dose, including emaciation and loss of weight. Concentrations of calcium and albumin were decreased, secondary to emaciation, and there was also a decrease in thymus weight in this group. Microscopic changes observed at this dose included atrophy of the parotid salivary gland (one male), exacerbated sexual immaturity (one male) and thymic involution (one male and both females).

Although there were compound-related decreases in the concentrations of the thyroid hormones triiodothyronine (T3), thyroxin (T4) and thyroid-stimulating hormone (TSH) in the groups at 400, 1600 and 6400 ppm (Table 19), these alterations were not considered to be biologically significant owing to the lack of accompanying abnormalities in thyroid weights or histological appearance of the thyroid gland in these dogs. The hormonal alterations were not considered to be of sufficient

Table 18. Incidence and average severity (figures in parenthesis) of microscopic findings in rats fed diets containing spirotetramat for 1 year

Parameter	Incidence (severity[a])			
	Dietary concentration (ppm)			
	0	250	3500	7500/12 000
Males (n = 25)				
Lungs, macrophages, alveolar	1 (1.0)	2 (1.0)	6 (1.0)*	11 (1.2)*
Testes, abnormal spermatozoa	0	0	0	2 (1.5)
Epididymides, exfoliated germ cells/debris	0	0	0	3 (2.0)
Females (n = 25)				
Lungs, macrophages, alveolar	5 (1.0)	3 (1.0)	2 (1.0)	21 (1.3)*

From Wahle (2005b)

* $p < 0.05$.

[a] Figures in parentheses indicate average severity, graded from 1 (minimal) to 5 (severe).

magnitude to produce biologically significant effects in the thyroid endocrine axis after 28 days of exposure.

The NOAEL was 1600 ppm, equal to 42 mg/kg bw per day, on the basis of decreases in body weight, decreased concentrations of calcium, albumin, T4, T3 and TSH, and decreased thymus weight and involution at 6400 ppm.

Table 19. Serum concentrations of thyroid hormones in male dogs (n = 2) given diets containing spirotetramat for 4 weeks

Dietary concentration (ppm)		Serum concentration of hormone (ng/ml)		
		−11 days	7 days	23 days
T3				
0	Mean	1.0	1.0	0.9
	SD	0.0	0.0	0.1
100	Mean	0.9	1.0*	0.9
	SD	0.0	0.0	0.1
400	Mean	0.9	0.8*	0.7
	SD	0.2	0.0	0.1
1600	Mean	1.1	0.7*	0.6
	SD	0.6	0.1	0.3
6400	Mean	0.9	0.4*	0.4
	SD	0.1	0.0	0.1
T4				
0	Mean	2.6	1.8	1.5
	SD	0.8	0.3	0.3
100	Mean	2.3	1.7	1.5
	SD	0.1	0.5	0.3
400	Mean	2.1	1.2	0.9*
	SD	0.1	0.7	0.1
1600	Mean	2.4	0.8	0.6*
	SD	0.7	0.3	0.0
6400	Mean	2.6	0.3	0.3*
	SD	0.2	0.2	0.3
TSH				
0	Mean	0.32	0.24	0.24
	SD	0.20	0.13	0.19
100	Mean	0.21	0.16	0.10
	SD	0.13	0.13	0.07
400	Mean	0.31	0.22	0.13
	SD	0.22	0.25	0.15
1600	Mean	0.21	0.09	0.12
	SD	0.01	0.06	0.04
6400	Mean	0.16	0.07	0.06
	SD	0.01	0.04	0.06

From Eigenberg (2004c)
SD, standard deviation; T3, triiodothyronine; T4, thyroxin; TSH, thyroid stimulating hormone.
* $p < 0.05$.

In a study that complied with GLP and for which a QA statement was provided, groups of four male and four female beagle dogs (aged 7–8 months) were given diets containing spirotetramat (purity, 97.6–97.8%) at a concentration of 0, 150, 300, 1200 or 4000/2500 ppm, corresponding to intakes of 5, 9, 33 and 81 mg/kg bw per day for males and 6, 10, 32 and 72 mg/kg bw per day for females (not including the highest dose), for 92–95 days. The group at the highest dose was given spirotetramat at 4000 ppm for 2 weeks, but this dose was subsequntly lowered to 2500 ppm owing to excessive

Table 20. Serum concentrations of thyroid hormones in female dogs (n = 2) given diets containing spirotetramat for 4 weeks

Dietary concentration (ppm)		Serum concentration of hormone (ng/ml)		
		−11 days	7 days	23 days
T3				
0	Mean	0.8	1.1	1.0
	SD	0.0	0.3	0.1
100	Mean	0.7	0.7	0.8
	SD	0.1	0.3	0.1
400	Mean	0.8	0.9	0.9
	SD	0.1	0.2	0.3
1600	Mean	1.1*	1.0	1.0
	SD	0.0	0.0	0.0
6400	Mean	0.8	0.5	0.4*
	SD	0.1	0.1	0.0
T4				
0	Mean	3.5	1.9	1.9
	SD	0.6	1.1	0.7
100	Mean	2.9	1.6	1.7
	SD	0.3	0.2	0.0
400	Mean	2.3	0.9	1.0
	SD	0.4	0.2	0.4
1600	Mean	3.5	1.2	1.4
	SD	0.6	0.2	0.1
6400	Mean	2.2	0.4	0.3*
	SD	0.7	0.2	0.0
TSH				
0	Mean	0.34	0.34	0.24
	SD	0.12	0.05	0.09
100	Mean	0.09	0.17	0.13
	SD	0.08	0.04	0.00
400	Mean	0.25	0.20	0.22
	SD	0.12	0.16	0.12
1600	Mean	0.24	0.19	0.29
	SD	0.13	0.13	0.19
6400	Mean	0.12	0.03	0.02
	SD	0.02	0.01	0.01

From Eigenberg (2004c)
SD, standard deviation; T3, triiodothyronine; T4, thyroxin; TSH, thyroid stimulating hormone.
*$p < 0.05$

weight loss. Clinical observations were conducted daily, food consumption was measured daily and body weights were taken weekly. Ophthalmological examinations were performed before exposure and before sacrifice. Clinical chemistry, haematology and urine-analysis measurements were taken once before exposure and during study weeks 4, 8 and 13. A gross necropsy was performed, organ weights were measured and tissues were examined microscopically.

No dogs were died or were sacrificed in extremis in this study.

During the first 2 weeks of the study, there was a compound-related reduction in body weight in the group at 4000 ppm. After week 2, when the dose of 4000 ppm was reduced to 2500 ppm, there was no compound-related effect on body weight. Although the females in this group began to gain body weight after the dietary concentration of test material was reduced, it was evident that they did not recover completely since the mean body weight at termination was still below the value before exposure. During the clinical assessments, one of these females was described as thin. Food-consumption values for this group were also depressed throughout the study.

Decline in values for erythrocyte parameters (erythrocyte count, haemoglobin concentration and erythrocyte volume fraction) were seen in females receiving spirotetramat at 2500 ppm on day 58; this decline continued throughout the remainder of the study and the difference between the values of these parameters in the group at 2500 ppm and the control group was statistically significant at day 84.

Gross examinations revealed that the thymus of one female at 2500 ppm was atrophied, or "reduced in size" as described in the histopathology report.

Compound-related decreases in concentrations of thyroid hormones were observed in the groups at 1200 and 2500 ppm. However, the alterations were not considered to be of a sufficient magnitude to produce biologically significant effects (i.e. no change in thyroid weight, no microscopic thyroid changes and no compensating increase in TSH).

The NOAEL was 1200 ppm, equal to 32 mg/kg bw per day, on the basis of body-weight loss and haematological effects in females at 4000/2500 ppm (Eigenberg, 2005).

In a short-term study that complied with GLP for which a QA statement was provided, groups of four male and four female beagle dogs (age 7–8 months) were given diets containing technical-grade spirotetramat (purity, 97.6–98.5%) at a concentration of 0 (concurrent vehicle control), 200, 600 or 1800 ppm, equivalent to 0, 6, 20 and 55 mg/kg bw per day for males, 0, 5, 19 and 48 mg/kg bw for females, respectively, for 1 year. Clinical observations were made daily. Food consumption was measured daily and body weights were taken weekly. Clinical chemistry, urine analysis and full blood count, including differentials, were performed for all dogs once before administration of the test substance. After initiation of dosing, haematological, clinical chemistry and urine-analysis data were collected from all dogs during study weeks 13, 25, 38 and 51. During study week 22, blood was collected from all dogs for evaluation of T4, T3 and TSH.. Ophthalmic examinations were performed before exposure and before sacrifice. A gross necropsy was performed, organ weights were measured and tissues were examined microscopically. The analysis of the results revealed that notable clinical findings, like dehydration, swelling, decreased activity and reactivity, seizures and ataxia were observed in a male at 1800 ppm. There were no unscheduled mortalities during the study. No compound-related effect on food consumption and body weight were observed. Haematologically, there were no toxicologically significant findings. There was a compound-related decrease in T4 for males and females at all doses and a decrease in T3 for males at all doses and for females at 1800 ppm. Two males showed a slight reduction in the size of the peripheral thyroid follicles that was considered to be compound-related. Thyroid follicular cells within the affected follicles in these two males did not differ from those of the controls. Despite the reduced serum concentrations of T3 and T4 at some time-points, no changes in thyroid weight and no compensating increases in TSH were seen at ≤ 600 ppm. Therefore

the decreases in thyroid hormones at ≤ 600 ppm were not considered to be toxicologically significant. The changes in serum concentrations of T4 and T3 are presented in Tables 21 and 22, respectively.

Gross necropsy and organ weights revealed a reduced thymus size and dilated brain in males at 600 and 1800 ppm. Thymus involution was graded as mild in one male at 600 ppm and as moderate in one male at 1800 ppm. Brain ventricular dilatation was also noted in females, but only at 600 ppm. Brain ventricular dilatation was seen at 600 ppm in one male (mild) and one female (moderate) as well as at 1800 ppm in one male (moderate); mild axonal degeneration was detected in one female at 1800 ppm. The brain ventricular dilatation was not accompanied by any clear histopathological

Table 21. Serum concentrations of thyroxin (T4) in dogs given diets containing spirotetramat for up to 1 year

Dietary concentration (ppm)	Mean serum concentration (µg/dl)					
	Day –5	Day 96	Day 155	Day 180	Day 271	Day 357
Males						
0	2.14	2.35	2.17	2.13	1.86	1.84
200	2.20	0.99	1.30	0.87	0.94	1.16
600	2.33	0.89	1.14	0.70	0.78	1.02*
1800	2.74	0.53*	1.01*	0.48*	0.33**	0.58**
Females						
0	2.44	1.83	2.56	2.18	1.80	2.88
200	2.57	1.19	1.76	1.38	1.23	1.79*
600	2.63	0.93*	1.24**	1.32	0.90*	1.11**
1800	2.37	0.69**	1.12**	0.51**	0.58**	0.85**

From Eigenberg (2006b)
* $p \leq 0.05$; ** $p \leq 0.01$

Table 22. Serum concentrations of triiodothyronine (T3) in dogs given diets containing spirotetramat for up to 1 year

Dietary concentration (ppm)	Mean serum concentration (µg/dl)					
	Day –5	Day 96	Day 155	Day 180	Day 271	Day 357
Males						
0	0.89	0.79	0.71	0.82	0.64	0.65
200	0.87	0.61	0.60	0.64**	0.49	0.57
600	0.91	0.68	0.62	0.61**	0.50	0.67
1800	0.95	0.48*	0.52*	0.52**	0.43*	0.46*
Females						
0	0.80	0.70	0.69	0.85	0.61	0.73
200	0.80	0.73	0.62	0.75	0.58	0.62
600	0.78	0.69	0.66	0.82	0.63	0.63
1800	0.87	0.62	0.59	0.61	0.50	0.61

From Eigenberg (2006b)
* $p \leq 0.05$; ** $p \leq 0.01$.

alteration. Organ weights were not affected by treatment. Mean thyroid absolute and relative organ weights were not statistically different from those of the controls. Mean absolute heart weight was statistically significantly lower ($p < 0.05$) at 1800 ppm in males; however, there was no significant difference in mean relative weight and correlative histopathological changes were not found. Absolute and relative thymus weights were low (although not statistically significantly) in males only at 600 ppm. Histopathology revealed that two males at 1800 ppm had a slight reduction in the size of the peripheral thyroid follicles. The Meeting concluded that the reduced serum concentrations of T3 and T4 at 600 ppm were not an adverse effect as these changes were inconsistent, and there was no TSH feedback response, no clinical findings indicating hypothyroidism, and no morphological changes in the thyroid.

The NOAEL was 200 ppm, equal to 5 mg/kg bw per day, on the basis of involution of the thymus. This NOAEL is conservative in view of the equivocal changes in thyroid hormones, and the brain ventricular dilatation of uncertain significance seen at 600 ppm (Eigenberg, 2006b).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity for which statements of compliance with GLP and QA were provided, groups of 55 male and 55 female CD-1 (CD-1 [ICR]/BR) mice (age approximately 9 weeks) were given diets containing spirotetramat (purity, 97.4–98.5%) at nominal dietary concentrations of 0, 70, 1700 or 7000 ppm for approximately 18 months. The highest dietary concentration of 7000 ppm was reduced to 6000 ppm from week 12 of the study in order to achieve an average intake of active ingredient of approximately 1000 mg/kg bw per day throughout the 18 months of exposure. The mean daily intake of spirotetramat over approximately 18 months at nominal dietary concentrations of 70, 1700 or 7000 ppm, respectively, was equal to 10.9, 263 and 1022 mg/kg bw per day for males and 13.7, 331 and 1319 mg/kg bw per day for females.

All test diets (including control) were consumed ad libitum at all times. The concentration of spirotetramat in the diet and the homogeneity and stability of spirotetramat as a dietary admixture were confirmed.

Body weight and food consumption determinations were conducted weekly for 13 weeks and once per month (at either 4 or 5 weeks, depending on the number of days in the month) thereafter; detailed clinical examinations of each animal were conducted weekly throughout the study. Observations for moribundity and mortality were performed at least once daily. Standard differential leukocyte and erythrocyte morphology end-points were evaluated from blood drawn via the orbital sinus of unfasted mice at approximately 12 and 18 months. All mice were given a post-mortem examination, which included documenting and saving all gross lesions, weighing designated organs, and collecting representative tissue specimens for histopathological evaluation.

After approximately 18 months of continuous dietary exposure to the test substance, the mice showed no evidence of a compound-induced toxicological response at any dose up to and including the limit dose. No evidence of a compound-induced neoplastic response was observed in any tissue examined.

The NOAEL was 7000 ppm, equal to 1022 mg/kg bw per day, on the basis of lack of findings at this, the highest dose tested (Wahle, 2006b).

Rats

In a study of carcinogenicity for which statements of compliance with GLP and QA were provided, groups of 55 male and 55 female Wistar Han rats (Crl:WI[Glx/BRL/Han]IGS BR) (age approximately 8 weeks) were given diets containing spirotetramat (purity, 97.4–98.5%) at nominal

dietary concentrations of 0, 250, 3500 or 7500 ppm (males)/12 000 ppm (females) for approximately 2 years. The mean daily intake of spirotetramat (mg spirotetramat/kg bw per day) over approximately 24 months at nominal dietary concentrations of 250, 3500 or 7500 (male)/12 000 (female) ppm, respectively, were 12.5, 169 and 373 mg/kg bw per day for males and 16.8, 229 and 823 mg/kg bw per day for females.

All test diets (including control) were consumed ad libitum at all times. The concentration of spirotetramat in the diet and the homogeneity and stability of spirotetramat as a dietary admixture were confirmed.

Body weight and food consumption determinations were conducted weekly for 13 weeks and once a month (at either 4 or 5 weeks, depending on the number of days in the month) thereafter; detailed clinical examinations of each rat were conducted weekly throughout the study. Observations for moribundity and mortality were performed at least once daily. Standard differential leukocyte and erythrocyte morphology end-points were evaluated from blood drawn via the orbital sinus from unfasted rats at approximately 12, 18 and 24 months. All rats were given a post-mortem examination, which included documenting and saving all gross lesions, weighing designated organs, and collecting representative tissue specimens for histopathological evaluation.

At a dietary concentration of 250 ppm, no effects attributable to exposure to spirotetramat were observed.

At dietary concentrations of 3500 ppm and greater there were decreases in absolute kidney weight and an increased incidence of renal tubular dilatation in males (0; 0; 8*; 19*, out of 55 rats, respectively, out of the 55 rats in each group; * $p < 0.05$) and females (0, 0, 16*, 42*, respectively, out of the 55 rats in each group; * $p < 0.05$).

At dietary concentrations of 7500 (males)/12000 (females) ppm, a decline in body weight of 10% and 14% was noted in males and females, respectively, based on final live body weight measured during week 103 (compared with controls). Clinical observations included an increased incidence of "body, scaly" (generally limited to the hind limbs) and yellow and brown staining in males and females (staining being generally located in the perigenital area and/or on the tail and being less prevalent in males). Gross observations were limited to an increased incidence of small discoloured and/or raised zones on the lungs of females. Organ-weight changes included decreased absolute kidney weight and increased relative lung weight in males and females.

Histopathological findings were noted in the kidneys, lungs, testes and epididymis, and the bile duct. In the kidney, findings included increases in the incidence of renal tubular dilatation in males at 3500 ppm and greater (0, 0, 8*, 19*, respectively, out of the 55 rats in each group; * $p < 0.05$) and females (0, 0, 16*, 42*, respectively, out of the 55 rats in each group; * $p < 0.05$). In the lungs, an increased incidence of alveolar macrophage accumulation and a complex of changes described as interstitial pneumonia were noted that was statistically significant at the highest dose. Both findings were described as a continuum in terms of morphological change (correlating with the gross lung observations observed in females at 12 000 ppm) and were evaluated together (males: 29, 32, 36, 47*; females: 24, 27, 32, 55*, respectively, out of the 55 rats in each group; * $p < 0.05$). Macrophage accumulation without additional change was coded as such. Interstitial pneumonia was diagnosed with the presence of one or more of the following lesions: presence of lymphocytes, cholesterol clefts, interstitial thickening of the alveolar septae by connective tissue, or increased alveolar pneumocytes and presence of occasional extravasation of erythrocytes (micro haemorrhage). The lung lesions described above were focal or multifocal in distribution and involved a very small overall portion of the lung tissue; most of the lung tissue was characterized as normal. The changes were of uncertain significance, possibly indicative of effects on the immune system at 7500 ppm. The effects on the lungs of males and females are shown in Table 23 and Table 24, respectively.

In the testes, an increased incidence of a generally slight morphological testicular change was noted (more subtle than distinct tubular degeneration), characterized by a depletion, asynchrony, and degeneration of latter-stage spermatids. In the epididymis, an increased incidence of immature/exfoliated germ cells/debris was observed in the lumen contents of the head, body and tail, which correlates with the testicular change. No morphological changes were observed in the anatomical structure of the epididymis tissue. These results are presented in Table 24.

In the bile duct, an increased incidence of hyperplasia/fibrosis with associated minimal periportal mononuclear-cell infiltrate was noted in females.

The NOAEL was 250 ppm, equivalent to 12.5 mg/kg bw per day, on the basis of a decrease in body-weight gain and structural changes in the kidneys at 3500 ppm. No evidence of a compound-induced neoplastic response was observed in any tissue examined (Wahle, 2006a).

Table 23. Lung effects in rats fed diets containing spirotetramat for 2 years

Effect	Dietary concentration (ppm)			
	0	250	3500	7500 (males)/12 000 (females)
Males				
No. of ratsa	40	33	44	35
Mean lung weight (g) ± SD	2.245 ± 0.309	2.362 ± 0.504	2.290 ± 0.426	2.420 ± 0.503
Mean relative lung weight ± SD	0.366 ± 0.059	0.402 ± 0.093	0.377 ± 0.074	0.435 * ± 0.111
No. of tissues examinedb	55	55	55	55
No. of tissues in which no abnormalities were detected	16	15	15	5
Abnormality:c				
Epithelialization	3 (2.0)	1 (1.0)	3 (2.3)	3 (2)
Alveolar macrophages	7 (1.3)	17 * (1.2)	17 * (1.4)	3 (1.0)
Granulomatous inflammation	3 (1.7)	1 (3.0)	3 (2.0)	6 (1.7)
Interstitial pneumonia	22 (1.6)	15 (1.7)	19 (1.7)	44 * (2.3)
Females				
No. of rats	38	39	37	42
Mean lung weight (g) ± SD	1.949 ± 0.478	1.899 ± 0.533	1.852 ± 0.392	1.934 ± 0.302
Mean relative lung weight	0.527 ± 0.138	0.492 ± 0.146	0.479 ± 0.111	0.600* ± 0.101
No. of tissues examined	55	55	55	55
No. abnormalities detected	27	25	17	0
Abnormality:a				
Epithelialization	—	—	2 (1.0)	—
Alveolar macrophages	20 (1.3)	14 (1.1)	18 (1.1)	3 (1.7)
Granulomatous inflammation	—	—	—	—
Interstitial pneumonia	4 (1.5)	13* (1.4)	14* (2.1)	52* (3.4)

From Wahle (2006a)

[a] Excluding ill or dead rats.

[b] All rats, including ill or dead rats.

[c] Figures in parentheses indicate average severity, graded from 1 (minimal) to 5 (severe).

* $p < 0.05$

2.4 Genotoxicity

Spirotetramat was evaluated for genotoxicity in a battery of studies of genotoxicity (five studies in vitro and three studies in vivo) (Table 25). All studies complied with GLP and QA statements were provided.

On the basis of the results of these studies, the Meeting concluded that spirotetramat has no mutagenic or genotoxic properties either in vivo or in vitro.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a dose range-finding study of reproductive toxicity, which complied with GLP and for a QA statement was provided, groups of 10 male and 10 female Wistar Hanover rats (age 14 weeks) were fed diets containing spirotetramat (purity, 97.5–97.8%) at nominal dietary concentrations of 0, 200, 500, 6000 or 10 000 ppm. The mean daily intake of spirotetramat is summarized in Table 26. All test diets (including control) were consumed ad libitum and the homogeneity and stability of spirotetramat as a dietary admixture was confirmed.

Body weight and food consumption were measured and detailed clinical examinations were conducted weekly throughout the study. Multiple reproductive parameters were evaluated. All rats were given a post-mortem examination that included documenting and saving all gross lesions, weighing designated organs and collecting representative tissue specimens for histopathological evaluation and sperm analysis. Additional investigations performed included developmental landmarks (vaginal patency and preputial separation) of the F_1 offspring, sperm analysis on F_1 males aged 8–9 weeks, histopathology of the epididymis and testes of the P-generation males, F_1 male weanlings aged 21 days, and F_1 males aged 8–9 weeks.

At the highest dietary concentration (10 000 ppm), no effects were observed on body weight, food consumption, or clinical observations among the P generation. Statistically significant declines (compared with the control group) in the absolute and relative weight of the cauda epididymis were observed. No pregnancies occurred and there were no offspring at this dietary concentration. Females did not have implantation sites. Sperm analysis revealed significant declines in the motility

Table 24. Microscopic findings in testes and epididymis of rats fed diets containing spirotetramat for 2 years

Findinga	Dietary concentration (ppm)			
	0	250	3500	7500
Testis				
No. of tissues examined	55	55	55	55
Degeneration	12 (3.4)	8 (3.5)	10 (2.9)	6 (3.2)
Spermatid degeneration, depletion, asynchrony	0	0	0	9 (1.2)*
Epididymis				
No. of tissues examined	55	55	54	54
Germ cell, exfoliated/debris	6 (2.2)	10 (1.9)	6 (1.8)	31 (2.5)*

From Wahle (2006a)

[a] Average severity is indicated in parentheses, graded from 1 (minimal) to 5 (severe).

* Statistically significant, $p < 0.05$.

Table 25. Results of studies of genotoxicity with spirotetramat

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium strains* TA1535, TA100, TA1537, TA98 and TA102	16–1581 µg/plate in DMSO	93.5–96.5	Negative ± S9	Herbold (2002a)
Reverse mutation[a]	*S. typhimurium* TA1535, TA100, TA1537, TA98 and TA102	16–1581 µg /plate in DMSO	95.2	Negative ± S9	Herbold (2006a)
Chromosomal aberration[b]	Chinese hamster V79 cells	10–50 µg/ml –S9 in DMSO 20–80 µg/ml +S9 in DMSO	93.5–96.5	Weakly positive	Herbold (2002b)
Chromosomal aberration	Chinese hamster V79 cells	0, 70 µg/ml –S9 in DMSO 0, 120 µg/ml +S9 in DMSO	98.6	Negative ± S9	Herbold (2003)
Forward mutation	HRPT locus in V79 cells (Chinese hamster lung cells)	2.5–80 µg/ml –S9 in DMSO 20–140 µg/ml +S9 in DMSO	93.5–96.5	Negative ± S9	Herbold (2002c)
In vivo					
Micronucleus formation	NMRI male mice (femoral marrow)	Two intraperitoneal doses at 0, 125, 250 or 500 mg/kg bw; sampling at 24 h after last dose	93.5–96.5	Negative	Herbold (2002d)
Chromosomal aberration	NMRI mice (bone marrow)	Single dose at 125, 250 or 500 mg/kg bw; sampling at 24 h and 48 h (highest dose) after last dose	92.7–93.2	Negative	Herbold (2003)
Unscheduled DNA synthesis	Male rat hepatocytes (Wistar)	A single oral dose at 0, 1000 or 2000 mg/kg bw Hepatocytes were prepared 4 h or 16 h after dosing.	92.7–93.2	Negative	Brendler-Schwaab (2003)

DMSO, dimethyl sulfoxide; S9, 9000 × g supernatant from rodent liver

[a] A repeat study.

[b] With one exception, cultures treated at the highest concentration showed statistically significant increases in numbers of metaphases with aberrations, with and without metabolic activation. However, these increases were very weak and may therefore have been induced by an impurity. In addition, metaphase quality at higher concentrations interfered with scoring, thus it may also be possible that the increased rates of chromosomal aberration were caused by a secondary non-genotoxic mechanism that was also responsible for reduced metaphase quality.

Table 26. Mean daily intake of spirotetramat by the parental generation in a dose range-finding study of reproductive toxicity in rats fed diets containing spirotetramat

Study phase	Mean daily intake (mg/kg bw per day)[a]			
	Dietary concentration (ppm)			
	200	500	6000	10 000
Males				
Premating	10.5	27.8	320.1	537.9
Females				
Premating	12.8	31.4	384.1	645.7
Gestation	12.9	31.9	393.9	—[b]
Lactation	26.7	74.4	831.0	—[b]

From Young (2006)

[a] Individual values were based on the means for each particular phase for each generation.

[b] No pregnancies occurred in the group at 10 000 ppm.

and percentage progression of sperm. A decline in epididymal counts was observed with no effect on testicular counts. There was an increase in the occurrence of morphologically abnormal sperm. Micropathology showed abnormal sperm in the epididymis (10 out of 10) and cauda epididymis (9 out of 10). The abnormal sperm consisted of what appeared to be the retention of the residual body to the tail of the spermatozoa, the severity of this change being graded as minimal to moderate. The results of sperm analysis/histopathology of epididymis and testes are shown in Table 27.

At 6000 ppm, parental females showed non-significant trends in body-weight declines during lactation. There were no treatment-related effects on food consumption or clinical observations, nor on of the reproductive parameters assessed. Among the F_1 offspring, a significant decline in pup weight occurred by day 21 (–13.9%) with total body-weight gain being significantly reduced (–14.7%) when compared with the control group. F_1 animals aged 8–9 weeks showed statistically significant declines in body weight. Slight declines in the motility and percentage progression of sperm were observed. There was an increase in the occurrence of morphologically abnormal sperm. Micropathology showed abnormal sperm in the epididymis (4 out of 15) and cauda epididymis (4 out of 14). The severity of the changes observed was considere to be minimal to moderate.

At dietary concentrations of 500 ppm and 200 ppm, no treatment-related effects were noted in the parental adults or the F_1 offspring.

Table 27. Effects on histopathology of the testes and epididymis and on sperm parameters in a dose range-finding study of reproductive toxicity in rats fed diets containing spirotetramat

Effect	Generation	Dietary concentration (ppm)				
		0	200	500	6000	10 000
Sperm analysis						
Motility (%)	P	74	74	79	75	31
	F1	90	90	88	76	
Progression (%)	P	57	54	58	52	14
	F1	69	64	63	56	
Testicular count (No./g)	P	99	128	107	111	98
	F1	82	75	73	73	
Epididymal count (No./g)	P	986	733	798	944	464
	F1	504	486	507	463	
Abnormal sperm a (No./200)	P	0.7	0.7	0.5	0.8	95.5
	F1	2.8	1.5	1.3	20.2	
Histopathology						
Abnormal epididymal sperm cells	P	0/10	ND	ND	0/10[b]	10/10
	F1	1/17	ND	ND	4/15	
Abnormal epididymal sperm cells (cauda)	P	0/10	ND	ND	0/10[b]	9/9
	F1	1/17	ND	ND	4/14	
Testes, degeneration	P	0/10	ND	ND	1/10[b]	0/10
	F1	0/17	ND	ND	0/15	

From Temerowski (2008)

Figures in italic type were considered to be treatment-related, although no statistical test for significance was used.

ND, not detected

[a] Presenting as amorphous heads.

[b] One P-generation male at 6000 ppm had degenerative testes and no sperm development, but this was considered to be not treatment-related. A single F_1 interim male control aged 8–9 weeks had a moderate number of abnormal sperm cells within epididymis and cauda epididymis).

The NOAEL for parental toxicity in P-generation males was 6000 ppm, equal to premating doses of 320.1 mg/kg bw per day, on the basis of reduced cauda epididymal weights and effects on sperm at 10 000 ppm, equal to 537.9 mg/kg bw per day. The highest dietary concentration (10 000 ppm) caused treatment-related effects on sperm cells of parental males and included decreased sperm motility and progression, decline in epididymal sperm count and increase in abnormal sperm in the epididymis and the cauda epididymis.

The NOAEL for parental toxicity in P-generation females was 500 ppm, equal to doses during lactation of 74.4 mg/kg bw per day, on the basis of slight declines in body-weight gain during lactation in the P-generation females at 6000 ppm,equal to 831 mg/kg bw per day.

The NOAEL for reproductive toxicity in P-generation males was 6000 ppm, equal to premating doses of 320.1 mg/kg bw per day, on the basis of decreased sperm motility and progression, decreased epididymal counts and increase in abnormal sperm in the epididymis and the cauda epididymis at 10 000 ppm, equal to 538 mg/kg bw per day. Other than the lack of pregnancies in females at 10 000 ppm, which was attributed to the sperm effects in males, there were no treatment-related reproductive effects observed in P-generation females during this pilot study.

The NOAEL for offspring toxicity was 500 ppm, equal to 74.4 mg/kg bw per day, on the basis of decreased body weights on day 21 of lactation at a dietary concentration of 6000 ppm, equal to 831 mg/kg bw per day. Decreases in pup weight corresponded with maternal body-weight decreases.

The NOAEL for male parental toxicity in F_1 males aged 8–9 weeks was 500 ppm on the basis of decreased terminal body weights at 6000 ppm.

The NOAEL for male reproductive toxicity in F_1 males aged 8–9 weeks was 500 ppm on the basis of decreased sperm motility and progression and an increase in abnormal sperm in the epididymis and the cauda epididymis at 6000 ppm (Young, 2006**a**).

In a two-generation study of reproductive toxicity, whichcomplied with GLP and for which a QA statement was provided, groups of 30 male and 30 female Wistar Hanover (Crl:WI[Glx/BRL/Han]IGS BR) rats (age 9–10 weeks) were fed diets containing spirotetramat (purity, 97.4–98.5%) at nominal doses of 0, 250, 1000 or 6000 ppm. The rats were exposed to the treated feed throughout the entire study. The parental animals (P and F_1 generations) were given test diets for 10 weeks before mating. Mating was accomplished by co-housing one female with one male for up to 14 consecutive days, with exceptions noted in the Protocol Amendments and Deviations. Approximately four to six rats from each treated group were co-housed daily beginning on the first day of the mating phase and continuing until all rats had been co-housed. During the mating phase, vaginal smears were taken each morning and examined for the presence of sperm and/or internal vaginal plug. Females found to be inseminated were placed in a polycarbonate nesting cage. The day on which insemination was observed in the vaginal smear was designated day 0 of gestation for that female. In order to evaluate females that may have been inseminated without exhibiting sperm in the vaginal smear or an internal vaginal plug, all remaining females were placed in polycarbonate nesting cages after the 14-day mating period. Selection of F_1 pups for evaluation of preputial separation and vaginal patency was made when the pups were aged 21 days. The F_2-generation pups were maintained until age 21 days.

Analysis of homogeneity, stability and achieved concentrations was reported. Mean daily intake of spirotetramat is shown in Table 28.

The exposure durations for rats in both generations were as follows: P-generation males, 16 weeks; P-generation females, 16–18 weeks; F_1-males, 24 weeks; F_1-females, 26_28 weeks; F_2-pups, 21 days.

Mortality checks were performed twice daily (morning and afternoon) during the working week and once daily at weekends and during holidays. Cage-side observations assessed mortality,

moribundity, behavioral changes, signs of difficult or prolonged delivery, and overt toxicity. In the event that possible clinical sign was observed during the cage-side evaluation, the rat was removed from the cage and a detailed assessment was conducted. A detailed evaluation of clinical signs was conducted once per week throughout the entire in-life phase of the study. Body weights and food consumption were measured and fresh feed provided once per week for males and females during the 10-week premating period with exceptions stated in the Protocol Amendments and Deviations. During the mating period and until sacrifice, body weights for the males were measured once per week. Body weights were also measured during the mating period for unmated females. Also during the mating period, fresh feed was provided for males and unmated females once per week, but food consumption was not measured. During gestation, dam body weights were measured on days 0, 6, 13 and 20, and fresh feed was provided and food consumption measured once per week. During lactation, dam body weights were measured on days 0, 4, 7, 14 and 21. Fresh feed was provided and food consumption measured once per week, with the exception of week 1 when food consumption was measured twice (days 0–4 and 4–7). The estrous cycle (determined by examining daily vaginal smears) was characterized for all P- and F_1-generation females during the 3 weeks before mating. Additionally, the estrous cycle stage was determined for all females immediately before termination. For all P- and F_1-generation males at termination, sperm was collected from one testes and one epididymis for enumeration of homogenization-resistant spermatids and cauda epididymal sperm reserves, respectively. In addition, an evaluation of morphology and motility was performed on sperm sampled from the distal portion (closest to the urethra) of the vas deferens. Sperm motility and counts were conducted using the IVOS (Integrated Visual Operating Systems, 1998). Morphology and testis counts were conducted on the control group and the group at the highest dose and in all treated groups for epididymal counts for the first generation. Morphology and counts were conducted on all treated groups for the second generation. Litters were examined after delivery and pups were sexed,

Table 28. Mean daily intake of spirotetramat in a two-geenration study in rats fed diets containing spirotetramat

Study phase	Mean daily intake (mg/kg bw per day)a		
	Dietary concentration (ppm)		
	250	1000	6000
Males			
P generation:			
Premating	17.2	70.7	419.3
F1-generation:			
Premating	19.3	79.5	486.7
Females			
P generation:			
Premating	20.0	82.5	484.7
Gestation	19.6	76.7	467.4
Lactation	39.4	162.9	895.7
F1 generation:			
Premating	21.7	90.3	539.5
Gestation	17.8	69.8	434.7
Lactation	39.4	161.0	930.6

From Young (2006)

[a] Individual values were based on the means for each particular phase for each generation.

examined for gross abnormalities and the number of stillborn pups and live pups recorded. Pup weight and external alterations were recorded on postnatal days 0, 4, 7, 14 and 21.

The analysis of the results showed that no treatment-related mortalities or clinical observations were observed at any dietary concentration tested in either generation. There were no treatment-related adverse effects at 250 ppm or 1000 ppm in either generation.

At 6000 ppm, the following observations were made (statistical significances are based on comparisons with the control group). In the P generation, males showed declines in body-weight gain during premating and females exhibited statistically significant declines in food consumption during lactation (days 0–21). F_1 offspring showed declines in pup weight (day 21) and pup-weight gain (days 4–21), which were considered to be secondary to maternal effects noted during lactation. Male and female F_1 pups (combined) aged 21 days were also reported to show an increase in relative brain weight and a decrease in absolute spleen weight, which were also considered to be secondary to pup-weight decreases.

At 6000 ppm, no compound-related effects were observed on the reproductive performance of the P generation.

F_1-generation adult males at 6000 ppm exhibited decreases in body weight from days 14–70 of premating, as well as declines in overall body-weight gain. F_1-generation females, during the premating phase, displayed decreases in overall body-weight gain when compared with the control group. Females also exhibited decreases in body-weight throughout gestation and in body weight and food consumption throughout lactation. In males and females, gross pathology and micropathology revealed terminal body-weight declines and microscopic changes in the kidney, consisting of dilated tubules with occasional proteinaceous material.

F_2 offspring at 6000 ppm also showed declines in pup weight (day 7–21) and pup-weight gain (days 4–21), which were considered to be secondary to maternal effects noted during lactation. Also observed in the pups aged 21 days (combined males and females) and considered to be secondary to pup-weight decreases was a decrease in absolute brain, thymus and spleen weight.

There were morphological effects on sperm (presenting as amorphous sperm heads) was noted in the F_1 generation; this was consistent with in the results of the dose range-finding study described above (Young, 2006). Variation in susceptibility was observed, as 9 out of 30 males exhibited a minimal effect (at least one amorphous sperm head was noted out of 200 viewed), but only one outlying male was affected to the extent that it compromised fertilizing capabilities. This same male showed abnormal sperm in the epididymis. Overall fertility in the group at this dose was not affected. No other compound-related reproductive findings were observed. The results of sperm analysis/histopathology of epididymis and testes are shown in Table 29.

The NOAEL for male parental toxicity was 1000 ppm, equal to premating doses of 70.7 mg/kg bw per day or 79.5 mg/kg bw per day in P- or F_1-generation males, respectively, on the basis of body-weight gain decreases in P-generation males at 6000 ppm, equal to 419.3 mg/kg bw per day, and body-weight gain decreases, decreased terminal body weights and increased renal multifocal tubular dilatation in F_1-generation males at 6000 ppm, equal to 486.7 mg/kg bw per day.

The NOAEL for female parental toxicity was 1000 ppm, equal to premating doses of 82.5 mg/kg bw per day or 90.3 mg/kg bw per day in P- or F_1-generation females, respectively, on the basis of reduced food consumption during lactation in P-generation females at 6000 ppm, equal to 484.7 mg/kg bw per day, and reduced food consumption during lactation, reduced body weights (end of premating), body-weight gain decreases (premating), decreased terminal body weights and increased renal multifocal tubular dilatation in F_1-generation females at 6000 ppm, equal to 539.5 mg/kg bw per day.

The NOAEL for reproductive toxicity was 1000 ppm in males, equal to premating doses of 79.5 mg/kg bw per day in the F_1 generation, on the basis of abnormal sperm cell morphology in F_1-generation males at 6000 ppm, equal to 486.7 mg/kg bw per day, which included one rat with abnor-

mal epididymal sperm cells and pronounced abnormal spermatozoa leading to compromised fertilizing capabilities and other animals (9 out of 30) showing one to four amorphous sperm heads out of 200 sperm cells viewed. Reproductive performance was not impaired at 6000 ppm in either generation.

The NOAEL for reproductive toxicity in females was 6000 ppm, equal to premating doses of 484.7 mg/kg bw per day or 539.5 mg/kg bw per day in the P or F_1 generation, respectively, on the basis of the absence of effects at 6000 ppm on mean duration of the estrus cycle, number of normally cycling females, mating fertility and gestation indices, mean duration of gestation, mating performance, vaginal opening, and litter parameters determined at birth (pup weight, total number of pups born, stillborn pups, viability index on lactation day 0, sex ratio and mean litter size).

The NOAEL for offspring toxicity was 1000 ppm on the basis of decreased body weights on day 21 of lactation and decreased body-weight gain in F_1 pups and decreased body weights on days 14 and 21 of lactation and decreased body-weight gain in F_2 pups at 6000 ppm. Pup-weight declines corresponded with maternal body weight and food consumption declines (Young, 2006).

(b) Developmental toxicity

Rats

In a pilot study of developmental toxicity that did not comply with standard GLP, groups of seven inseminated female Wistar Hsd Cpb:WU rats were given spirotetramat (purity, 98.8%) at a

Table 29. Effects on histopathology of the testes and epididymis and on sperm parameters in a two-generation study of reproductive toxicity in rats fed diets containing spirotetramat

Effect	Generation	Dietary concentration (ppm)				
		0	250	1000	6000	Historical controls
Sperm analysis						
Motility (%)	P	84	83	84	85	76–90
	F1	81	83	84	80	—
Progression (%)	P	59	59	61	62	48–68
	F1	57	60	59	56	—
Testicular count (No./g)	P	90	—	—	87	64–81
	F1	88	90	90	79	—
Epididymal count (No./g)	P	623	563	482*	472*	234–431
	F1	517	490	520	497	—
Abnormal sperma (No./200)	P	2.0	—	—	2.8	0.8–3.1
	F1	2.9	2.6	1.9	8.9	—
Histopathology						
Abnormal epididymal sperm cells	P	0/30	—	—	0/30	—
	F1	0/30	—	—	1/30	
Abnormal epididymal sperm cells (cauda)	P	0/30	—	—	0/30	—
	F1	0/30	—	—	1/30	
Testes, degeneration	P	0/30	—	—	0/30	—
	F1	0/30	—	—	0/30	

From Young (2006)

[a] Presenting as amorphous heads: one male at 250 ppm with aspermia (considered to be not treatment-related), animal evaluated owing to gross necropsy findings (Temerowski, 2006).

* $p < 0.05$.

Figures in italic type are considered to be treatment-related.

daily oral dose of 0, 50, 200, 800 or 1000 mg/kg bw per day (dose volume, 10 ml/kg bw) by gavage in 0.5% aqueous carboxymethylcellulose from day 6 to day 19 post coitum. The groups at 200 mg/kg bw per day and 1000 mg/kg bw per day comprised 11 rats and 8 rats, respectively. For better clarification of toxic effects, seven additional females were added later to the group at 1000 mg/kg bw per day. The fetuses were delivered by caesarean section on day 20 post coitum. Investigations were performed on general tolerance of the test compound and its effect on intrauterine development.

Treatment-related effects included maternal effects at 800 mg/kg bw per day (body-weight loss, impaired body-weight development) and at 1000 mg/kg bw per day (respiratory findings, piloerection, body-weight loss, impaired body-weight gain, increased urination, light-coloured faeces). Effects on intrauterine development could not be completely excluded at 200 mg/kg bw per day (possible marginal reduction in fetal weight), and were also evident at 800 mg/kg bw (reduced placental and possibly fetal weight, retarded ossification, wavy ribs). Effects were clearly observed at 1000 mg/kg bw per day (distinctly reduced fetal and placental weight, necrotic placental borders, retarded ossification, wavy and 14th ribs and possible marginal increase in the incidence of common malformations) (Klaus, 2001).

In a study of developmental toxicity that complied with GLP and for which a QA statement was provided, groups of 25 inseminated female Wistar Hsd Cpb:WU rats were given spirotetramat (purity, 99.0% *cis* isomer) at a daily oral dose of 0, 20, 140 or 1000 mg/kg bw per day by gavage in 0.5% aqueous carboxymethylcellulose from day 6 to day 19 post coitum. The fetuses were delivered by caesarean section on day 20 of gestation. Investigations were performed on general tolerance of the test compound and its effect on intrauterine development. The study was performed in compliance with international guidelines (OECD guideline No.414, 2001; US EPA Health Effects Test Guidelines OPPTS 870.3700, 1998; Japanese MAFF guidelines of 2000 as amended in 2001and EEC Commission Directive 88/302/EEC, 1988).

Mortality, appearance and behaviour were not affected by treatment with spirotetramat at doses of up to 1000 mg/kg bw per day. Treatment-related effects at 1000 mg/kg bw per day comprised impaired feed intake after start of treatment and up to the end of study, transient marginal body-weight loss together with impaired body-weight gain and reduced final body weight and carcass weight. Light-coloured faeces were observed in the group at 1000 mg/kg bw per day, although this finding might be related to the large amount of white test substance given.

Necropsy revealed no treatment-related findings at doses of up to 1000 mg/kg bw per day. A marginal reduction in placental weight together with a more distinct reduction in fetal weight was observed in the group at 1000 mg/kg bw per day. External, visceral and skeletal evaluation of fetuses revealed a slightly increased number of fetuses and litters with generally common non-specific malformations at the maternally toxic dose of 1000 mg/kg bw per day. A potential of spirotetramat to induce a specific type of malformation was not deduced from these findings. Overall incidence and type of malformations at 20 and 140 mg/kg bw per day did not indicate a treatment-related effect. External fetal deviations were not observed in this study and the incidence and type of visceral deviations of fetuses was not affected by treatment with spirotetramat at doses of up to 1000 mg/kg bw per day. Effects on food consumption and body-weight gain were, however, observed at 140 mg/kg bw per day.

Skeletal, including cartilaginous, tissue evaluation of fetuses revealed retarded ossification at 1000 mg/kg bw per day, together with an increased incidence of skeletal variations (wavy ribs, 14th ribs, combined osseous and cartilaginous findings) (Table 30). Furthermore, retarded ossification of single localizations and increased incidence of wavy ribs were seen in the groups at 140 and 20 mg/kg bw per day, without a clear dose–response relationship. On the basis of the results of an additional supplementary study with spirotetramat (described below; Klaus, 2004a), which revealed no indication for a treatment-related increase in the incidence of wavy ribs at doses up to 140 mg/kg bw per

day and no evidence for effects on degree of ossification at up to 140 mg/kg bw per day, the Meeting excluded the possibility of a treatment-related effect on ossification and incidence of wavy ribs at a dose up to 140 mg/kg bw per day in the present study.

The NOAEL for systemic maternal toxicity was 140 mg/kg bw per day on the basis of treatment-related effects at 1000 mg/kg bw per day (impaired feed intake, transient marginal body-weight loss together with impaired body-weight gain and reduced final body weight and carcass weight).

The NOAEL for intrauterine development was 140 mg/kg bw per day on the basis of ossification at 1000 mg/kg bw per day, together with an increased incidence of skeletal variations (wavy ribs, 14th ribs, combined osseous and cartilaginous findings) (Klaus, 2004b).

On the basis of the results of a study of developmental toxicity in rats given spirotetramat at a dose of 20, 140 or 1000 mg/kg bw per day described above in Klaus (2004b) in which equivocal retarded ossification of single localizations and equivocal increased incidence of wavy ribs were seen at 140 and 20 mg/kg bw per day without a clear dose–response relationship, an additional study of developmental toxicity was performed for clarification of results. The study complied with GLP and

Table 30. External visceral and skeletal malformations in fetuses in a study of developmental toxicity in rats given spirotetramat by gavage

Malformationa	Dose (mg/kg bw per day)			
	0	20	140	1000
Cleft palate	—	—	—	1
Microphthalmia (eye rudiment flat/eyeball reduced in size/eye socket reduced in size), unilateral	1	1	—	1
Anophthalmia (eye rudiment flat/eyeball missing/eye socket reduced in size), unilateral	1	1	—	—
Upper jaw shortened, macroglossia, domed head, all bones of fore-and hindlimbs, of skull and vertebral column dysplastic	—	—	1	—
Lobe of thyroid gland absent	3 (2)	—	1	—
Atrial septal defect of the heart	1	1	—	1
Coarctation of aortic arch between left carotid and left subclavian arteries, ascending aorta reduced in size, left subclavian artery arises from descending aorta	—	—	—	1
Dysplasia of forelimb bones (scapula, humerus, radius and/or ulna)	1	2 (2)	—	4 (4)
Supernumerary lumbar vertebra	—	—	—	1
First sacral vertebral arch has the shape of a lumbar vertebral arch in the osseous and the cartilaginous part and the cartilaginous part is not fused with the cartilaginous processus transversus of the secnd sacral vertebral arch, pelvis shifted caudally	—	—	—	3 (3)
Number of fetuses per group	247	301	253	270
Number of fetuses with malformations	7	5	2	12
Malformed fetuses per group (%)	2.83	1.66	0.79	4.44
Number of litters per group	20	24	23	22
Number of litters with malformations	4	4	2	9
Malformed litters per group (%)	20.00	16.67	8.70	40.91

From Klaus (2004b)

[a] The number of litters affected is given in parentheses.

a QA statement was provided. Groups of 25 inseminated female Wistar rats were given spirotetramat (purity, 99.1%) as a daily oral dose at 0, 10, 35 or 140 mg/kg bw per day by gavage in 0.5% aqueous carboxymethylcellulose from day 6 to day 19 post coitum. The fetuses were delivered by caesarean section on day 20 of gestation. Investigations were performed on general tolerance of the test compound, including haematology, clinical chemistry, liver weight and histopathology of liver, as well as the effect of spirotetramat on intrauterine development.

Treatment-related maternal effects with respect to mortality, clinical signs including appearance and amount of excreta (urine, faeces), food intake, body-weight development, haematology parameters, liver weight, necropsy findings and histopathology of liver were not observed at doses of up to 140 mg/kg bw per day. Treatment-related effects on reproductive parameters, i.e. gestation rate, post-implantation loss, litter size, placental weight and appearance, fetal weight and fetal sex distribution were not evident at doses of up to 140 mg/kg bw per day. The incidence and type of fetal malformations were unaffected by treatment at doses of up to 140 mg/kg bw and a teratogenic potential of spirotetramat at doses of up to 140 mg/kg bw per day was not evident. Meaningful fetal external or visceral deviations (findings other than malformations) were not evident at doses of up to 140 mg/kg bw per day. Evaluation for degree of fetal skeletal ossification and incidence of skeletal variations, including evaluation of cartilaginous structures, revealed no clear evidence for treatment-related effects at doses of up to 140 mg/kg bw per day. The incidence of wavy ribs was not affected at doses of up to 140 mg/kg bw per day. Summarizing and evaluating all data investigated, the following no-observed adverse-effect-levels (NOAEL) were determined:

The NOAEL for maternal toxicity was 140 mg/kg bw per day. The NOAEL for developmental toxicity was 140 mg/kg bw per day (Klaus, 2004a).

Rabbits

In a pilot study of developmental toxicity that was not conducted in accordance with standard GLP, groups of three mated female Himalayan rabbits were given spirotetramat as a daily oral dose at 0, 5, 25, 100, 160, 250 or 500 mg/kg bw per day by gavage in 0.5% carboxymethylcellulose in demineralized water from day 6 to day 28 post coitum. The fetuses were delivered by caesarean section on day 29 post coitum. Investigations were performed on the general tolerance of the test compound and its effect on intrauterine development (including external and visceral evaluation of the fetuses).

One female at 500 mg/kg bw per day died on day 10 post coitum. The remaining two females of this group and one female at 250 mg/kg bw per day were killed for humane reasons on day 10 or 18 post coitum (500 mg/kg bw per day) or day 23 post coitum (250 mg/kg bw per day), presenting wounds in the region of the head, throat or forelimbs. Another female at 250 mg/kg bw per day was killed after abortion on day 24 post coitum. Hypoactivity (lying on side) on a single day occurred in one female in each of the groups at 500 and 250 mg/kg bw per day as well as in the female that aborted in the group at 250 mg/kg bw per day, which also showed laboured breathing and convulsions. Cold ears, severely decreased or no feed intakes and distinct to severe body-weight loss (up to 343 g) also occurred in females at 500 and 250 mg/kg bw per day.

Necropsy revealed hardened fatty tissue in the abdominal cavity and at the border of the pancreas and round depressions in the gastric mucosa in one female at 500 mg/kg bw per day; an enlarged gall bladder in one female and gaseous contents in the stomach and intestine as well as haemorrhages in the renal capsule in an additional female at 250 mg/kg bw per day. Cold ears occurred for several days in all females at 160 mg/kg bw per day. Feed intakes were transiently distinctly to severely decreased in two females at 160 mg/kg bw per day, these rabbits also showing moderate to distinct body-weight loss (191 g and 273 g). Gross necropsy did not reveal treatment-related findings at doses of up to 160 mg/kg bw per day.

The gestation rate in the group at 250 mg/kg bw per day was decreased by one abortion and by one total resorption. Owing to this abortion, total resorption, early sacrifice or death of all females in

the groups at 250 and 500 mg/kg bw per day, the evaluation of the remaining reproduction parameters was limited to doses up to and including 160 mg/kg bw per day. The resorption rate and the number of fetuses as well as fetal sex distribution were unaffected at doses up to and including 160 mg/kg bw per day. Placental and fetal weights in the group at 160 mg/kg bw per day were slightly decreased when compared with the control group, which was, however, most likely to be caused by the incidentally higher litter sizes in the group at 160 mg/kg bw per day rather than a treatment-related effect. A definitive assessment is, however, not possible owing to the low number of females in this pilot study.

The isolated malformations seen in one fetus at 100 mg/kg bw per day (malposition of forelimb) and one fetus in each group at 25 and 160 mg/kg bw per day (cardiac ventricular septal defect with/without truncus arteriosus) are considered incidental as these malformations are known to be common findings in the strain of rabbits used. Thus maternal toxicity was evident at 160 mg/kg bw per day and was severe at doses of 250 mg/kg bw per day and above including death or sacrifice in moribund condition. The abortion and the total resorption at 250 mg/kg bw per day were considered to be caused by maternal toxicity rather than being a specific effect on reproduction. Developmental toxicity (slightly decreased fetal weights) could not be excluded at 160 mg/kg bw per day as this dose revealed maternal toxicity.

The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of body-weight loss, impaired body-weight development, decreased food consumption and cold ears at 160 mg/kg bw per day. The NOAEL for developmental toxicity was 100 mg/kg bw per day on the basis of marginally reduced fetal and placental weights at 160 mg/kg bw per day. No detailed raw data were provided in the study report (Holzum, 2001).

In a study of developmental toxicity that complied with GLP and for which a QA statement was provided, groups of 22 pregnant Himalayan (CHBB:HM) rabbits were given spirotetramat (purity, 98.9%) as a daily oral dose at 0, 10, 40 (24 rabbits) or 160 (30 rabbits) mg/kg bw per day by gavage in 0.5% aqueous carboxymethyl cellulose from day 6 to 28 post coitum. Stability, homogeneity and dose concentrations were confirmed analytically. On day 29 of gestation, the fetuses were delivered by caesarean section.

One female at 160 mg/kg bw per day was found dead after having shown reddish excretion and soft faeces and five females had to be sacrificed in moribund condition after having shown severely reduced to zero feed intake, severe body-weight loss, cold ears, alopecia, reduced or no faeces, diarrhoea (in one female) and soft and light-coloured faeces, reddish excretion, decreased water intake and urination and discoloured urine most probably indicating concentration of urine. Females that had to be sacrificed in moribund condition showed fluid and/or gaseous contents in the caecum, discoloured liver and mottled gall bladder at necropsy. Abortion was observed most likely as a consequence of maternal toxicity in two other females at 160 mg/kg bw per day and in one female at 40 mg/kg bw per day that showed clinical symptoms, impaired feed intake and body-weight loss before abortion; i.e. symptoms comparable to those of the females that had to be sacrificed in moribund condition. The remaining females at 160 mg/kg bw per day had cold ears, alopecia and soft, mucoid and light-coloured faeces. The abortion in the group at 40 mg/kg bw per day was considered to be incidental. Post-implantation loss in the remaining females, and the number of fetuses as well as placental weight and appearance, fetal weight and fetal sex distribution were unaffected by treatment at doses of up to 160 mg/kg bw per day.

Fetal malformations seen were different in type, scattered between the different doses and revealed the highest total incidence of malformations in the group at 10 mg/kg bw per day, lying well within the normal range of scattering for the strain of rabbits used and showing no dose-dependency. Malformations of cartilaginous parts of ribs, cardiac ventricular septal defects and malpositioned forelimbs (one of the most common malformations in the rabbit strain used) seen in a number of

fetuses were not dose-related and single malformations that appeared only once in the group at 160 mg/kg bw per day (domed head together with encephalomeningocele, cleft palate, microphthalmia) were different in type and comparable with data on historical controls. Thus a teratogenic effect of spirotetramat was excluded at doses of up to 160 mg/kg bw per day. Apart from a possibly increase in the incidence of fetuses with distinct liver lobulation in the group at 160 mg/kg bw per day, a treatment-related effect on fetal external and visceral deviations (findings other than malformations) was not found at doses of up to 160 mg/kg bw per day. Fetal skeletal including cartilaginous tissue evaluation revealed **no** treatment-related effects at a dose up to 160 mg/kg bw per day. Findings for the progeny (abortions at 40 and 160 mg/kg bw per day, distinct fetal liver lobulation at 160 mg/kg bw per day) only occurred at doses with signs of distinct (40 mg/kg bw per day) to severe maternal toxicity.

The NOAEL for systemic maternal toxicity was 40 mg/kg bw per day on the basis of abortion, clinical signs, impaired food and water consumption and body-weight loss at 160 mg/kg bw per day (the LOAEL). The NOAEL for developmental toxicity was is 160 mg/kg bw per day, the highest dose tested (Klaus, 2004c).

2.6 Special studies

(a) Study screening for acute oral neurotoxicity

In a study screening for acute oral neurotoxicity, which complied with GLP and for which a QA statement was provided, groups of 12 male and 12 female nonfasted young adult Wistar Crl:WI(Glx/BRL/Han) IGS BR rats (age 9 weeks) were given spirotetramat (purity, 97.8–98.5%) as a single oral dose at 0 (vehicle), 200, 500 or 2000 mg/kg bw by gavage in 0.5% methylcellulose/0.4% Tween 80 in deionized water (adjusted to pH 4 with acetic acid to enhance stability) and administered in a dosing volume of 10 ml/kg. Since there were compound-related effects at 200 mg/kg bw, a follow-up study was conducted under the same conditions and providing nominal doses of 0 (vehicle), 50, 100 and 500 mg/kg bw to verify the findings at 500 mg/kg bw. The following observations and measurements were included in the initial study: mortality checks, clinical observations, body-weight measurements, a functional observational battery (FOB), automated measurements of activity (figure-eight maze), a gross necropsy, brain weight, measurements and microscopic examination of skeletal muscle, peripheral nerves, eyes (with optic nerves), and tissues from the central nervous system. Observations and measurements included in the follow-up study consisted of mortality checks, clinical observations, body-weight measurements, a FOB and figure-eight maze.

On the basis of analytical results, the actual doses of spirotetramat for the initial study were 0, 182, 515 and 1930 mg/kg bw for males and females and actual doses for the follow-up study were 0, 47.4, 99.8 and 523 mg/kg bw for males and females.

No compound-related deaths occurred at any dose.

In the initial study, compound-related clinical signs were evident at all doses. Urine stains were observed at all doses and were considered to be related to treatment. Perianal staining was observed in males at the two higher doses (one in each group) but not at the lowest dose or in females at any dose. These signs were evident on day 0 and generally resolved within 1–4 days after treatment. For the follow-up study, urine stains were evident in females at 500 mg/kg bw, but not at lower doses or in males at any dose. This sign was first observed on day 0 and resolved within 1–4 days after treatment. Body weight was not affected by treatment in males or females at any dose.

For the FOB in the initial study, urine stains were observed in males (one each) at the lowest and intermediate dose. There were no findings related to treatment observed in males at the highest dose or females at any dose. In the follow-up study, there were no treatment-related findings at any dose in males or females. Measures of motor and locomotor activity in the initial study were

significantly reduced in males at the highest dose (48% and 64%, respectively) and females (40% and 65%, respectively). At 500 mg/kg bw, motor activity was reduced (32%) and locomotor activity was significantly reduced (44%) in males only. Locomotor activity was slightly reduced (29%) in males at the lowest dose. In addition, motor and locomotor activity was reduced in males at several time-points at 500 and 2000 mg/kg bw and for locomotor activity in males at the lowest dose. A similar trend was evident in females at 500 and 2000 mg/kg bw (locomotor activity only at the intermediate dose), but the difference from controls was not statistically significant. In the follow-up study, motor and locomotor activity was slightly reduced (21% and 30%, respectively) in males at 500 mg/kg, but not at lower doses or in females at any dose. Motor and locomotor activity was reduced at several time-points in both sexes at 500 mg/kg bw.

There were no compound-related effects on days 7 or 14 after treatment and habituation was not affected by treatment on any day, at any dose. There were no compound-related gross lesions in males or females at any dose. Brain weight was not affected by treatment in males or females at any dose. Compound-related microscopic lesions were not evident in males or females at the highest dose.

To summarize, an acute oral dose of spirotetramat produced evidence of toxicity in males and females at all doses (200, 500 and 2000 mg/kg bw) in the initial study. Evidence of toxicity was limited to clinical signs (urine staining at all doses, perianal staining in males at the intermediate and highest dose) and decreased activity in the figure-eight maze (females at the intermediate and highest dose and males at all doses) beginning on the day of treatment and with complete recovery by day 7. There was no evidence of neurotoxicity at any dose and there were no compound-related gross or microscopic lesions at a limit dose of 2000 mg/kg bw. A follow-up study confirmed findings in the initial study at 500 mg/kg bw, with no compound-related effects at lower doses. The results of these studies are summarized in Tables 31–37.

The NOAEL was 100 mg/kg bw (Gilmore et al., 2005).

(b) Mechanism of action

Two mechanistic studies were conducted to identify the time of onset and location of the first visible effects on the rat testes, and to determine the metabolite responsible for the testicular/sperm toxicity attributable to spirotetramat.

Table 31. Clinical observations in initial and follow-up studies of acute neurotoxicity in rats given spirotetramat by gavage

Observation [a]	Dose (mg/kg bw)					
	0	50	100	200	500	2000
Males (n = 12)						
Urine stain						
Initial study	0	—	—	1 (days 0–1, 7) [a]	3 (day 0)	5 (days 0–2)
Follow-up study	0	0	0	—	0	—
Perianal stain						
Initial study	0	—	—	—	1 (day 0)	1 (day 0)
Females (n = 12)						
Urine stain						
Initial study	0	—	—	1 (day 0)	6 (days 0–1)	6 (days 0–3)
Follow-up study	0	0	0	—	6 (days 0–3)	—

From Gilmore et al. (2005)

[a] Range of days on which the observation was made is given in parentheses.

In a study to identify the primary target cell(s) of spirotetramat in male rat testes and epididymis that complied with GLP and for which a QA statement was provided, groups of 32 male rats (Crl:WI[Glx/BRL/Hans]IGS BR strain)(aged approximately 13 weeks) were given spirotetramat (purity, 97.2%) at a dose of 0 (vehicle only) or 1000 mg/kg bw per day by gavage in an aqueous solution of 0.5% methylcellulose 400 for 3, 10, 21 or 41 days (eight rats per scheduled sacrifice time). Clinical signs were recorded daily, body weight and food consumption was measured at least weekly. A detailed physical examination was performed once during the acclimatization phase and weekly throughout the study. Serial sacrifices were performed on days 3, 10, 21 and 41, approximately 4 h after the last dosing. All rats were necropsied, selected organs weighed and a range of tissues was taken, fixed and examined microscopically. In addition, after sacrifice, sperm from the right epididymis was collected for sperm enumeration and morphology assessment.

There was one treatment-related death of a rat at 1000 mg/kg bw per day on day 31. This rat showed only increased salivation on days 17 and 21 and a slightly reduced body-weight gain between days 21 and 28. Several of the remaining rats showed clinical signs of toxicity on skin or fur (lesion, piloerection, generalized or localized soiled fur), movement or behaviour (reduced motor activity,

Table 32. Mean motor and locomotor activity for initial study of acute neurotoxicity in rats given spirotetramat by gavage

Test day	Mean total activity counts per session ± standard deviation			
	Dose (mg/kg bw)			
	0	200	500	2000
Males (n = 12)				
Motor activity:				
Day −7	416 ± 219	318 ± 121	385 ± 138	383 ± 135
Day 1	516 ± 182	546 ± 420	350 ± 141 (−32%)a	270* ± 83 (−48%)[a]
Day 7	493 ± 170	573 ± 70	409 ± 79	459 ± 128
Day 14	410 ± 118	429 ± 125	346 ± 103	374 ± 96
Locomotor activity:				
Day −7	265 ± 172	202 ± 93	239 ± 91	238 ± 93
Day 1	312 ± 140	222 ± 102 (−29%)[a]	174* ± 80 (−44%)[a]	113* ± 39 (−64%)[a]
Day 7	307 ± 126	270 ± 65	233 ± 45	271 ± 75
Day 14	236 ± 82	227 ± 83	175 ± 43	221 ± 80
Females (n = 12)				
Motor activity:				
Day −7	508 ± 169	399 ± 166	567 ± 136	539 ± 116
Day 1	491 ± 142	443 ± 162	444 ± 110	294* ± 135 (−40%)[a]
Day 7	520 ± 246	450 ± 294	530 ± 130	477 ± 160
Day 14	409 ± 160	494 ± 190	428 ± 209	469 ± 177
Locomotor activity:				
Day −7	329 ± 108	220* ± 122	363 ± 117	345 ± 81
Day 1	292 ± 90	262 ± 112	251 ± 104	102* ± 47 (−65%)[a]
Day 7	294 ± 105	256 ± 170	315 ± 76	261 ± 88
Day 14	239 ± 108	303 ± 163	263 ± 153	240 ± 95

From Gilmore et al. (2005)

[a] Percentage decrease compared with control values is shown in parentheses.

* Significantly different from control, $p \leq 0.05$.

hyper-reactivity, tremors), mouth (increased salivation), respiration (noisy, coughing) and general appearance (general pallor, wasted, hunched posture) for a limited period. A mean body-weight loss was observed between days 1 and 10 and a body weight remained static between days 10 and 15. Thereafter, mean body-weight gain per day was slightly lower than values for the controls. As a consequence, mean body weight progressively decreased throughout the study from 2% after 3 days of treatment to 12% at study termination on day 41 ($p < 0.01$ or $p < 0.05$), compared with controls. Mean food consumption was decreased by 22% ($p < 0.01$) from days 1–8, when compared with the control group. Subsequently, mean food consumption was slightly reduced by 6–10% from days 8–22, or was comparable to the control values thereafter.

Table 33. Mean motor activity in an initial study of acute neurotoxicity in rats given spirotetramat by gavage

Interval	Mean motor activity (No. of movements/10 min interval) ± standard deviation							
	Dose (mg/kg bw)							
	0	200	500	2000	0	200	500	2000
	Males				Females			
Day −7								
1	96 ± 27	87 ± 15	93 ± 27	90 ± 15	104 ± 19	92 ± 29	116 ± 13	106 ± 20
2	80 ± 48	76 ± 31	80 ± 20	78 ± 37	89 ± 29	78 ± 50	107 ± 30	93 ± 19
3	77 ± 49	68 ± 39	80 ± 30	85 ± 25	98 ± 46	73 ± 42	104 ± 31	100 ± 24
4	65 ± 44	65 ± 44	58 ± 29	65 ± 26	87 ± 48	65 ± 35	107 ± 35	94 ± 25
5	58 ± 49	19 ± 21	52 ± 47	43 ± 38	70 ± 37	46 ± 50	80 ± 41	88 ± 35
6	39 ± 28	4* ± 97	22 ± 31	22 ± 31	61 ± 49	45 ± 43	53 ± 44	59 ± 43
Day 1								
1	115 ± 31	118 ± 48	91 ± 29	80* ± 13	118 ± 36	114 ± 21	105 ± 24	76 ± 30
2	105 ± 34	109 ± 88	73 ± 22	64 ± 55	95 ± 49	86 ± 27	78 ± 30	50 ± ± 26
3	113 ± 48	97 ± 67	55* ± 22	34* ± 17	93 ± 36	73 ± 40	76 ± 24	50 ± 31
4	82 ± 36	97 ± 86	54 ± 29	28* ± 19	79 ± 35	70 ± 38	74 ± 30	33 ± 29
5	65 ± 47	75 ± 103	41 ± 27	37 ± 24	63 ± 28	52 ± 42	52 ± 22	49 ± 40
6	36 ± 36	50 ± 75	35 ± 34	26 ± 18	43 ± 38	49 ± 39	60 ± 29	35 ± 28
Day 7								
1	120 ± 38	124 ± 32	117 ± 22	121 ± 21	126 ± 37	132 ± 51	143 ± 34	138 ± 23
2	98 ± 29	87 ± 27	80 ± 16	86 ± 17	105 ± 29	98 ± 57	115 ± 31	108 ± 39
3	87 ± 36	104 ± 82	77 ± 15	76 ± 29	81 ± 34	73 ± 52	93 ± 19	85 ± 37
4	68 ± 40	146 ± 275	67 ± 26	72 ± 35	63 ± 27	53 ± 60	70 ± 33	66 ± 36
5	63 ± 31	56 ± 24	40 ± 29	61 ± 43	50 ± 33	48 ± 52	67 ± 62	46 ± 40
6	57 ± 39	56 ± 30	28 ± 23	44 ± 36	96 ± 223	47 ± 50	43 ± 37	34 ± 29
Day 14								
1	115 ± 36	122 ± 27	115 ± 29	107 ± 24	121 ± 46	136 ± 44	124 ± 60	128 ± 42
2	83 ± 30	90 ± 24	76 ± 17	81 ± 10	88 ± 37	108 ± 45	92 ± 51	101 ± 39
3	78 ± 24	77 ± 21	65 ± 34	73 ± 20	70 ± 33	86 ± 46	69 ± 38	77 ± 48
4	59 ± 34	61 ± 30	41 ± 18	45 ± 17	52 ± 34	69 ± 53	58 ± 43	59 ± 40
5	42 ± 27	38 ± 33	27 ± 32	30 ± 20	43 ± 26	47 ± 30	49 ± 37	57 ± 44
6	33 ± 21	41 ± 35	21 ± 24	39 ± 34	36 ± 27	49 ± 18	36 ± 45	48 ± 32

From Gilmore et al. (2005)
* $p \leq 0.05$.

Sperm analysis after 3 or 10 days of treatment revealed no treatment-related changes in the numbers of spermatozoa (absolute and relative to the epididymis weight) or in the frequency of all types of abnormal spermatozoa. After 21 days, the numbers of spermatozoa were still comparable to the control values, but the frequency of all types of abnormal spermatozoa was slightly increased in the treated group (6.4% vs 2.5% in the control group, $p < 0.01$). After 41 days of treatment, the absolute and relative numbers of spermatozoa were markedly decreased by 77% and 68% ($p < 0.01$), respectively, compared with controls. In association, changes in sperm morphology were more marked,

Table 34. Mean locomotor activity in an initial study of acute neurotoxicity in rats given spirotetramat by gavage

Interval	Mean locomotor activity (No. of movements/10 min interval) ± standard deviation							
	Dose (mg/kg bw)							
	0	200	500	2000	0	200	500	2000
	Males (n = 12)				Females (n = 12)			
Day −7								
1	62 ± 24	57 ± 14	63 ± 23	60 ± 12	70 ± 17	55 ± 18	77 ± 15	70 ± 13
2	51 ± 39	46 ± 24	46 ± 14	48 ± 25	55 ± 21	44 ± 36	69 ± 28	54 ± 14
3	52 ± 39	44 ± 27	52 ± 22	52 ± 21	64 ± 32	43 ± 34	64 ± 26	60 ± 23
4	42 ± 34	41 ± 29	34 ± 19	42 ± 19	59 ± 35	36 ± 25	71 ± 31	66 ± 24
5	38 ± 36	11 ± 14	31 ± 29	25 ± 24	45 ± 24	25 ± 26	51 ± 27	55 ± 23
6	19 ± 18	3 ± 6	13 ± 20	11 ± 16	36 ± 27	18 ± 21	31 ± 29	40 ± 29
Day 1								
1	76 ± 21	65 ± 23	54* ± 15	45* ± 14	78 ± 19	70 ± 12	62 ± 20	35 ± 16
2	64 ± 30	44 ± 24	34* ± 14	19* ± 9	53 ± 22	51 ± 20	47 ± 27	18 ± 11
3	68 ± 38	44* ± 24	28* ± 16	14* ± 10	51 ± 21	43 ± 24	44 ± 22	14 ± 8
4	50 ± 29	39 ± 26	29 ± 22	13* ± 11	45 ± 21	39 ± 26	41 ± 23	11 ± 8
5	34 ± 30	18 ± 17	15* ± 14	12* ± 14	39 ± 20	32 ± 30	26 ± 15	15 ± 12
6	20 ± 25	13 ± 19	14 ± 16	9 ± 7	26 ± 27	28 ± 27	30 ± 18	9 ± 6
Day 7								
1	84 ± 9	86 ± 25	74 ± 16	78 ± 10	86 ± 27	85 ± 32	99 ± 25	84 ± 19
2	61 ± 24	50 ± 22	45 ± 13	50 ± 9	72 ± 23	57 ± 38	74 ± 26	63 ± 28
3	54 ± 30	43 ± 12	46 ± 12	45 ± 18	56 ± 26	39 ± 27	58 ± 20	45 ± 21
4	41 ± 27	39 ± 14	36 ± 16	43 ± 25	36 ± 18	29 ± 34	38 ± 27	34 ± 20
5	38 ± 21	29 ± 14	20 ± 16	35 ± 28	26 ± 23	23 ± 25	26 ± 22	21 ± 19
6	30 ± 27	24 ± 15	13 ± 11	21 ± 21	18 ± 20	23 ± 30	20 ± 17	15 ± 15
Day 14								
1	76 ± 21	72 ± 18	66 ± 14	71 ± 21	82 ± 35	92 ± 41	89 ± 47	82 ± 29
2	48 ± 22	48 ± 13	38 ± 11	46 ± 7	49 ± 24	67 ± 37	60 ± 40	57 ± 22
3	43 ± 18	40 ± 16	32 ± 16	41 ± 19	41 ± 20	52 ± 41	41 ± 26	40 ± 21
4	31 ± 22	31 ± 19	18 ± 9	27 ± 14	25 ± 22	35 ± 39	32 ± 28	26 ± 19
5	21 ± 15	18 ± 21	12 ± 14	16 ± 13	24 ± 16	29 ± 23	25 ± 21	14 ± 14
6	17 ± 15	19 ± 22	9 ± 11	20 ± 21	18 ± 19	28 ± 12	17 ± 23	21 ± 22

From Gilmore et al. (2005)
* $p \leq 0.05$.

Table 35. Mean motor and locomotor activity during the follow-up study of acute neurotoxicity in rats given spirotetramat by gavage

Test day	Mean total activity counts per session ± standard deviation			
	Dose (mg/kg per day)			
	0	50	100	500
Males (n = 12)				
Motor activity:				
Day −7	404 ± 160	460 ± 100	383 ± 100	382 ± 160
Day 1	373 ± 124	387 ± 111	349 ± 97	296 ± 74 (−21%)
Locomotor activity:				
Day −7	276 ± 128	323 ± 82	242 ± 76	240 ± 104
Day 1	257 ± 100	274 ± 84	228 ± 73	179 ± 50 (−30%)
Females (n = 12)				
Motor activity:				
Day −7	474 ± 222	483 ± 109	539 ± 162	443 ± 151
Day 1	440 ± 178	485 ± 192	470 ± 189	375 ± 175
Locomotor activity:				
Day −7	319 ± 171	306 ± 97	350 ± 112	312 ± 122
Day 1	293 ± 131	323 ± 142	321 ± 152	236 ± 131

From Gilmore et al. (2005)

[a] Percentage decrease compared with control values is shown in parentheses.

Table- 36. Mean motor activity in the follow-up study of acute neurotoxicity in rats given spirotetramat by gavage

Interval	Mean motor activity (No. of movements/10 min interval) ± standard deviation							
	Dose (mg/kg bw)							
	0	50	100	500	0	50	100	500
	Males (*n* = 12)				Females (*n* = 12)			
Day –7								
1	96 ± 32	105 ± 18	101 ± 16	104 ± 37	98 ± 23	97 ± 16	115 ± 18	103 ± 19
2	85 ± 30	107 ± 27	77 ± 17	87 ± 32	82 ± 32	89 ± 24	92 ± 30	80 ± 21
3	75 ± 24	98 ± 21	67 ± 25	73 ± 36	84 ± 45	94 ± 31	88 ± 35	82 ± 39
4	65 ± 43	68 ± 39	60 ± 32	49 ± 31	76 ± 48	94 ± 28	93 ± 37	75 ± 41
5	47 ± 40	48 ± 34	45 ± 30	40 ± 34	73 ± 47	58 ± 29	78 ± 28	61 ± 38
6	36 ± 30	35 ± 36	34 ± 24	29 ± 31	60 ± 50	52 ± 34	72 ± 38	42 ± 35
Day 1								
1	92 ± 28	95 ± 23	81 ± 15	84 ± 22	129 ± 50	119 ± 29	118 ± 22	87* ± 32
2	82 ± 26	78 ± 20	74 ± 16	58 ± 19	99 ± 36	119 ± 27	93 ± 21	77 ± 25
3	75 ± 20	72 ± 32	58 ± 30	47 ± 21	77 ± 37	87 ± 41	88 ± 35	63 ± 38
4	55 ± 21	60 ± 20	55 ± 25	38 ± 22	59 ± 43	71 ± 46	62 ± 48	58 ± 38
5	41 ± 25	49 ± 22	51 ± 24	34 ± 18	45 ± 35	48 ± 40	63 ± 54	40 ± 34
6	29 ± 24	32 ± 30	31 ± 24	35 ± 29	32 ± 29	41 ± 48	46 ± 37	50 ± 43

From Gilmore et al. (2005)

* Significantly different from controls, $p \leq 0.05$.

as the frequency of total abnormal spermatozoa reached 72.0% in the treated group compared with 3.8% in the control group ($p < 0.01$).

At necropsy, mean terminal body weight was comparable to values for the control group at sacrifice on day 3, but was reduced by 6–12% at days 10, 21 and 41. No toxicologically-relevant changes were noted in the testes, epididymis or prostate weights at days 3, 10 or 21. At day 41, the prostate gland weight was unaffected by treatment, but mean absolute and relative testes and epididymis weights were statistically significantly reduced by 11–26%. At the macroscopic examination, no treatment-related gross pathology changes were detected after 3, 10 and 21 days of treatment. However, after 41 days, small epididymis was observed in all rats, together with a small prostate gland in 2 out of 7 rats and small and/or soft testis in 6 out of 7 rats.

Microscopic examination revealed adverse treatment-related changes in the seminiferous tubules and epididymis after 21 days of treatment. In the testes, marked degenerating elongating spermatids (steps 9–14 of the maturation cycle) were found in 8 out of 8 rats treatedwith spirotetramat, together with multinucleated giant spermatids in 2 out of 8 rats and moderate to marked degenerating round spermatids in 4 out of 8 rats. These morphological findings identified after 21 days of treatment are consistent with a treatment-related effect in round spermatids or late stage spermatocytes. In the epididymis, slight to moderate increase of intraluminal abnormal aberrant cells were found in all treated rats after 21 days of treatment, as a consequence of degenerating spermatids observed in the testis. At final sacrifice on day 41, slight to severe degenerating elongating spermatids (steps 9–14) were found in 7 out of7 treated rats, together with loss of elongating spermatids (steps 9–19) in 5 out of 7 treated rats. Multinucleated giant spermatids in 4 out of 7 rats and marked degenerating round spermatids (around steps 7–8) in 5 out of 7 rats were also observed. These morphological findings in the testes confirm what was observed after 21 days of treatment and may be consistent with a

Table 37. Mean locomotor activity in the follow-up study of acute neurotoxicity in rats given spirotetramat by gavage

Interval	Mean locomotor activity (No. of movements/10 min interval) ± standard deviation							
	Dose (mg/kg bw)							
	0	50	100	500	0	50	100	500
	Males (n = 12)				Females (n = 12)			
Day −7								
1	73 ± 28	81 ± 17	73 ± 15	70 ± 17	68 ± 19	65 ± 13	76 ± 13	77 ± 16
2	56 ± 28	78 ± 27	49 ± 15	60 ± 27	57 ± 25	55 ± 21	57 ± 22	53 ± 17
3	51 ± 22	72 ± 22	46 ± 19	48 ± 24	56 ± 37	64 ± 23	55 ± 24	57 ± 34
4	44 ± 33	45 ± 26	36 ± 21	31 ± 22	54 ± 38	60 ± 25	64 ± 28	52 ± 30
5	30 ± 30	28 ± 24	26 ± 20	20 ± 24	48 ± 31	34 ± 23	54 ± 22	45 ± 32
6	21 ± 21	20 ± 26	13 ± 13	10 ± 17	37 ± 36	28 ± 24	43 ± 21	29 ± 26
Day 1								
1	73 ± 23	76 ± 18	62 ± 12	61 ± 14	99 ± 39	89 ± 27	88 ± 20	64* ± 25
2	57 ± 23	57 ± 16	50 ± 15	37 ± 15	71 ± 28	79 ± 21	64 ± 20	48 ± 20
3	51 ± 19	53 ± 25	37 ± 22	30 ± 17	53 ± 29	58 ± 33	61 ± 29	39 ± 28
4	38 ± 19	42 ± 15	32 ± 18	23 ± 14	33 ± 30	45 ± 37	42 ± 39	33 ± 27
5	22 ± 18	31 ± 17	28 ± 16	16 ± 10	23 ± 27	28 ± 28	44 ± 40	22 ± 22
6	16 ± 15	17 ± 19	17 ± 16	12 ± 10	15 ± 15	23 ± 29	22 ± 22	30 ± 28

From Gilmore et al. (2005)
* $p \leq 0.05$.

treatment-related effect in round spermatids. As a consequence of degenerating spermatids observed in the testis,a marked increase of intraluminal aberrant cells associated with a moderate to marked oligospermia were found in the epididymis in all treated rats on day 41. In conclusion, spirotetramat at 1000 mg/kg bw per day over at least 21 days induced treatment-related effects in round spermatids or late stage spermatocytes and subsequent changes in the testis and epididymis (Kennel, 2005).

The testicular/sperm toxicity attributed to spirotetramat was investigated using the enol metabolite of spirotetramat. The structure of spirotetramat consists of two parts – an enol entity and an acyl chain. The enol is the major metabolite of spirotetramat in rats whereas the acyl chain of spirotetramat aids the penetration of spirotetramat into the plant and is cleaved once in the plant. As the structure of the acyl chain resembles that of the established testicular toxicant, methoxy acetic acid, it was proposed that this chain may be responsible for the testicular toxicity of spirotetramat. However, as this moiety is chemically unstable, the enol metabolite of spirotetramat was investigated. This study did not comply with GLP nor was a QA statement provided.

Groups of five male rats (strain Rj:WI(IOPS HANS) (aged 12–13 weeks) were given the metabolite spirotetramat-enol (ourity, 96.8; a beige powder) at a dose of 0 (vehicle only) or 800 mg/kg bw per day by gavage in 0.5% methylcellulose in a dose volume of 5 ml/kg for 21 days. Clinical signs and body weights were recorded daily. Food consumption was measured weekly. A detailed physical examination was performed once during the acclimatization period and weekly throughout the study. All rats were sacrificed 4 h after the last of 21 doses. All rats were necropsied and selected organs were excised and weighed. Both testes and the left epididymis from each rat were fixed and examined microscopically. Sperm from the right epididymis were collected for sperm enumeration and morphological assessment. The dose of 800 mg/kg bw per day of spirotetramat-enol used in this study was chosen as it is equivalent to a dose of 1000 mg/kg bw per day of the parent compound, spirotetramat, which has been used in a previous investigative study described above (Kennel, 2005) in which testicular and sperm toxicity were observed. The exposure period selected was 21 days, as significant testicular and sperm effects were first recorded at this time-point in the previous study investigating the parent compound.

As clinical signs of toxicity, body-weight effects and a treatment-related death were attributd to administration of the parent compound, a preliminary test was performed to evaluate the toxicity of spirotetramat-enol, the results of which were not described.

Oral exposure to spirotetramat-enol for 21 days did not induce any treatment-related mortality. However, a range of clinical signs was observed, particularly in the later stages of the study. These signs included localized soiled fur (anogenital and mouth regions), increased salivation and reduced motor activity. A mean body-weight loss was observed on days 2 ($p \leq 0.01$, when compared with controls), 14, 16, 18 ($p \leq 0.01$ when compared with controls) and 20. The mean body-weight gain on all other days of study was similar to control values. This resulted in the overall body-weight gain being only 22% of that of the controls. This was not, however, statistically significant due to the large standard deviations for both groups. The mean body weight was consistently, though not statistically significantly reduced compared with controls, from 1.3% on day 2 to 7% on the last day of dosing. Mean food consumption was reduced by 14.2% ($p \leq 0.05$) during days 1–8 and by 8.5% during days 8–15. The mean food consumption was similar to control values during the last week of exposure.

At necropsy, the mean terminal body weight of the treated group was lower (–8%, not statistically significant) when compared with controls. The mean relative testes weights were statistically significantly higher ($p \leq 0.05$), but this change mainly correlated with the lower terminal body weights. Microscopic examination of the seminiferous tubules and the epididymis revealed a treatment-related change after 21 days of treatment. Sloughing of germ cells associated with degenerating elongating spermatids was found in all rats treated with spirotetramat-enol. In the left epididymis, exfoliated germ cells were found in all treated rats, as a consequence of degenerating spermatids observed in the testes. Sperm analysis revealed a slight increase in the absolute numbers of spermatozoa (5%, not

statistically significant) whereas there was a slight decrease in the relative (to the epididymal weight) numbers (7.9%, not statistically significant) after 21 days of treatment. The frequency of total abnormal spermatozoa was significantly increased ($p \leq 0.05$) after 21 days of treatment (14.9% vs 3.2% in the control group). Most of these abnormalities were either isolated heads of normal morphology or spermatozoa with a normal head but possessing an abnormal mid-piece.

In conclusion, oral exposure of male rats to spirotetramat-enol at a dose of 800 mg/kg bw for 21 days induced treatment-related effects in the testes and epididymis and induced an increase in the frequency of spermatozoa with an aberrant morphology. The effects observed in this study are similar, both in terms of observations and magnitude of responses, to those recorded for a previous study in which the parent compound, spirotetramat, was assessed under similar conditions (Kennel, 2005). Based on the results of this study, the Meeting concluded that the testicular/sperm toxicity of the spirotetramat is unlikely to be due to its acyl chain but rather to its major metabolite i.e. spirotetramat-enol (Tinwell, 2006).

(c) Studies with metabolites

(i) Acute toxicity

Studies of acute oral toxicity have been carried out with a number of metabolites, as summarized in Table 38.

(ii) Genotoxicity

Studies of genotoxicity have been carried out with a number of metabolites, as summarized in Table 39.

Table 38. Acute oral toxicity with metabolites of spirotetramat in female Wistar rats

Metabolite	Strain	Purity (%)	LD50 (mg/kg bw)	Reference[a]
Spirotetramat-cis-ketohydroxy	HsdCpb:Wu	98.7	> 2000	Schuengel (2005a)
Spirotetramat-desmethyl-ketohydroxy	HsdCpb:Wu	94.6	> 2000	Schuengel (2006a)
Spirotetramat-mono-hydroxy	HsdCpb:Wu	98.41	> 2000	Schuengel (2005b)
Spirotetramat-di-hydroxy	HsdCpb:Wu	94.5.	> 2000	Schuengel (2006b)

[a] All studies complied with good laboratory practice and statements of quality assurance were provided.

Table-39. Studies of genotoxicity with metabolites of spirotetramat in vitro

Metabolite	End-point	Test object	Concentration/dose	Purity (%)	Result	Reference[a]
Spirotetramat-*cis*-ketohydroxy	Point mutation	*S. typhimurium* TA1535, TA100, TA1537, TA98 and TA102	≤ 1581 µg per plate ± S9	98.7	Negative	Herbold (2005a)
Spirotetramat-desmethyl-ketohydroxy	Point mutation	*S. typhimurium* TA1535, TA100, TA1537, TA98 and TA102	≤ 500 µg per plate ± S9	94.6	Negative	Wirnitzer (2006)
Spirotetramat-mono-hydroxy	Point mutation	*S. typhimurium* TA1535, TA100, TA1537, TA98 and TA102	≤ 5000 µg per plate ± S9	98.41	Negative	Herbold (2005b)
Spirotetramat-di-hydroxy	Point mutation	*S. typhimurium* TA1535, TA100, TA1537, TA98 and TA102	≤ 5000 µg per plate ± S9	94.5	Negative	Herbold (2006b)

S9, 9000 × *g* supernatant from rodent liver

[a] All studies complied with good laboratory practice and statements of quality assurance were provided.

(iii) [Azaspirodecenyl-3-^{14}C]- spirotetramat-enol-glucoside: study of absorption, distribution, excretion and metabolism in the rat

Spirotetramat-enol-glucoside was a main metabolite of spirotetramat in a study of the metabolism of spirotetramat in lettuce and hence was included in the plant-residue method (Hass et al., 2004). Spirotetramat-enol-glucoside was detected in fruiting and leafy crops in European residue trials (unpublished results). Because it was an uncommon metabolite in the study of absorption, distribution, excretion and metabolism in rats, an additional study was conducted in order to investigate the absorption, distribution, excretion and metabolism of spirotetramat-enol-glucoside in male rats. The study complied with GLP and a QA statement was provided.

One male rat was given a single dose of spirotetramat-enolglucoside (radiolabelled with ^{14}C in the 3-position of the spiro-ring of the molecule) at a target dose of 0.1 mg/kg bw by oral gavage in aqueous saline solution. Plasma, urine and faeces were collected at various times after dosing. Skin, gastrointestinal tract and carcass were sampled at sacrifice.

Of the administered dose, 98.3% was recovered from measurement of the total radioactivity in urine and faeces as well as in skin, gastrointestinal tract and carcass at sacrifice, as shown in Table 40.

The radiolabelled spirotetramat-enol-glucoside was rapidly absorbed from the gastrointestinal tract of the male rat. Absorption started immediately after oral dosing. Excretion was rapid and was almost complete by 24 h after dosing. The radiolabelled residues in skin, gastrointestinal tract and carcass of the rat were determined at sacrifice, 48 h after dosing. A negligible amount of radiolabel was found in skin and gastrointestinal tract. Thequantity of radiolabel in the carcass amounted to 1% of the administered dose.

The test item and its metabolites were identified in this study by reverse-phase HPLC. The identification rate was high and amounted to 93% of the administered dose. Spirotetramat-enol was the main metabolite in excreta (about 64% of the administered dose). Minor metabolites were spirotetramat-desmethyl-enol (about 5%) and spirotetramat-ketohydroxy (about 3%). Unchanged spirotetramat-enol-glucoside was detected in excreta (about 21% of the administered dose), while 20.7% of the administered dose was found in the faeces. Only one minor, polar, metabolite (0.5% of the administered dose) in the faeces was not identified (Klempner, 2006d).

(iv) [Azaspirodecane-3-14C] spirotetramat-ketohydroxy: study of absorption, distribution, excretion and metabolism in the rat

Spirotetramat-ketohydroxy is a metabolite of spirotetramat that was found in the studies of metabolism in target crops (apple, lettuce and cotton), and identified as a main metabolite in cotton (Sur et

Table 40. Recovery of radiolabel from a rat given a single dose of spirotetramat-enolglucoside by gavage

Sample	Recovery (% of administered dose)
Urine	53.3
Faeces	43.7
Total excreted	97.0
Skin	0.09
Sum of organs	0.99
Body without gastrointestinal tract	1.08
Gastrointestinal tract	0.11
Total body	1.19
Balance	98.3

From Klempner (2006d)

al., 2005). Spirotetramat-ketohydroxy was also detected in a confined rotational-crop study, where it was the main metabolite in Swiss chard and turnip roots. In the study described here, which complied with GLP and for which a QA statement was provided, a group of four male Wistar rats was given a single dose of spirotetramat-ketohydroxy (radiolabelled with ^{14}C at the carbon at position 3 in the azaspirodecane ring (radiochemical purity > 99%) at a target dose of 2 mg/kg bw by oral gavage in aqueous Tragacanth®. The rats were killed 48 h after dosing. Total radioactivity, including parent compound and/or metabolites, was determined in the excreta (urine and faeces) as well as in organs and tissues. Investigations on metabolites were performed with selected samples of urine and with faecal extracts.

About 99% of the administered dose was recovered from the urine and faeces and in organs and tissues. Radiolabelled spirotetramat-ketohydroxy was rapidly absorbed from the gastrointestinal tract of male rats, absorption starting immediately after oral dosing. Although no exact value for the absorption rate could be derived from these observations, it was assumed that a high proportion of the administered dose was absorbed and systemically available, in view of this behaviour and the fact that the test item was intensively metabolized showing the same metabolic pattern in the urine and faeces.

Excretion was rapid and was almost complete 24 h after dosing. Of the total recovery of 98.6%, 54.5% of the dose was excreted via the urine and 44.1% via the faeces.

The radiolabelled residues in the organs and tissues of the rats were determined at sacrifice, 48 h after dosing. Negligible amounts of radiolabel were found in the skin, liver and gastrointestinal tract, showing that the elimination of the compound-related radioactivity was nearly complete. The concentrations of residues in all other organs and tissues were very low (< 0.01 ppm, or < LOD).

For elucidation of the metabolism, urine samples and faecal extracts were analysed by HPLC with radiodetection and by LC/MS and LC/MS-MS. The identification rate was high, amounting to 86% of the administered dose. The test item was detected as a minor quantity of < 1% of the administered dose in faeces only. Spirotetramat-ketohydroxy was completely metabolized, forming numerous metabolites. The first and most important metabolic reaction was the oxidative demethylation of the cyclohexyl-*O*-methyl group to the respective alcohol (spirotetramat-desmethylketohydroxy). The two isomers of spirotetramat-desmethyl-ketohydroxy were the main components in excreta accounting for 15% of the administered dose. All other identified metabolites were further degradation products of spirotetramat-desmethyl-ketohydroxy. Most were different mono-, di- and tri-oxygenated metabolites leading in a first metabolic transformation to the corresponding hydroxy metabolites. A second oxidative transformation (formal loss of two hydrogen atoms) gave the corresponding keto-metabolites with or without additional double-bond formation. The oxygenated metabolites were classified in three groups to simplify evaluation and quantification: mono-hydroxy, di-hydroxy and tri-hydroxy metabolites of spirotetramat-desmethyl-ketohydroxy. About 63% of the dose comprised the various different mono- and di-hydroxy metabolites of spirotetramat-desmethyl-ketohydroxy. Tri-hydroxy metabolites were of minor importance. Only one metabolite of this group was detected, accounting for about 3% of the administered dose. Conjugation with e.g. glucuronic acid and sulfate, was detected for few metabolites only and at low quantities. Cleavage of the azaspirodecane ring of spirotetramat-desmethylketohydroxy was found to a minor extent, as shown by two metabolites: spirotetramat-desmethyl-mandelic acid-amide and spirotetramat-desmethyl-glyoxyilic amide. The two metabolites comprised ≤ 2.8% of the administered dose. Only five minor metabolites (all < 1.6% of the administered dose) were not identified, but were characterized by their HPLC elution behaviour (Klempner, 2006e).

(d) Comparative study of in-vitro dermal absorption of [^{14}C]spirotetramat in human and rat skin

In a study that complied with GLP and for which a QA statement was provided, the dermal penetration of [^{14}C]spirotetramat in a soluble concentrate (SC240) formulation through rat and

human dermatomed skin was investigated at nominal concentrations of 240, 1.5 and 0.05 mg/ml, for the highest, intermediate and lowest dose formulations, respectively. The mean percentage of [^{14}C]spirotetramat considered to be directly absorbed over 24 h from the formulation providing the highest dose was 0.10% and 0.29% for human and rat skin, respectively. The mean percentage of [^{14}C]spirotetramat considered to be potentially absorbable over 24 h from the formulation providing the highest dose was 0.41% and 2.90% for human and rat skin, respectively, yielding a factor difference of 7.1 between the two species for the undiluted product. For the formulation providing the intermediate dose, the mean percentage directly absorbed was 0.07% and 1.49% for human and rat skin, respectively. For the formulation providing the intermediate dose, the mean percentage potentially absorbable was 1.41% and 11.76% for human and rat skin, respectively, yielding a factor difference of 8.3 between the two species for the spray dilution. For the formulation providing the lowest dose, the mean percentage directly absorbed was 0.24% and 6.99% for human and rat skin, respectively. For the formulation providing the lowest dose, the mean percentage potentially absorbable was 11.47% and 16.32% for human and rat skin, respectively, yielding a factor difference of 1.4 between the two species for the spray dilution (Capt, 2006).

3. Observations in humans

3.1 Assessment of skin sensitization in workers handling spirotetramat

Spirotetramat, which is known from experiments in laboratory animals to be a dermal sensitizer, has caused two proven cases of type-4 sensitization (allergic contact dermatitis) in workers handling the undiluted active ingredient.

In 2004, two cases of type-4 skin sensitization were observed and confirmed by patch testing in two workers handling the active ingredient spirotetramat during development. One worker had been mixing active ingredient into the animal feed for toxicology testing, and the other had prepared test formulations containing active ingredient. In both cases, the facial skin was involved, and in both cases the use of personal protective equipment was not satisfactory. Symptoms were reddening, itching, swelling and eczema of the facial skin in the preorbital area.

To rule out further cases among employees and to possibly improve protection, a questionnaire survey was performed in nine countries. The partipants included staff involved in research, development, industrial operations and health care of the manufacturer. Participation (which was voluntary in nature) was satisfactory (175 out of 269 workers; 65%). The questionnaire ruled out sensitization to spirotetramat in 170 out of of 175 employees (97%). Five employees (3%) required further assessment owing to vague indications of possible sensitization. In three of these cases, sensitization could be ruled out by an occupational physician; in a fourth case sensitization was ruled out by an occupational dermatologist. Patch testing for sensitization to spirotetramat was proposed in one case, but the employee declined this offer. The joint judgement of the two physicians involved was that sensitization was unlikely, yet not definitely ruled out. Although no further cases of sensitization have been detected, technical and personal protective-equipment protection must be maintained. Occupational medical surveillance for exposed workers should continue (Steffens, 2005).

3.2 Occupational medical experiences with spirotetramat

Occupational medical surveillance of 12 workers exposed to spirotetramat, performed yearly on a routine basis as reported by the manufacturer and not directly related to exposures, did not reveal any unwanted effects in the workers except the two previously mentioned proven cases of type-4 sensitization. The medical examinations included history, full physical examination with orientating neurological status (reflexes, sensibility and coordination) and skin status. The technical

examination and laboratory investigation included lung function, electrocardiogram/ergometry, vision testing, audiometry, chest X-ray, sonography (if necessary); blood sugar random (BSR), full blood count, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, glucose, creatinine, cholesterol, and urine status. All the workers used personal safety measures to the manufacturers' standards, full mask with filter ABEK-P3, protective gloves for chemicals, chemical-resistant suit and safety glasses (Kehrig, 2006).

3.3 Literature survey

A number of literature databases were investigated concerning the following issues in regard to spirotetramat:

- Reports on clinical cases and poisoning incidents;
- Epidemiological studies;
- Clinical signs and symptoms of poisoning.

None of the databases contained publications on incidents of human poisoning with spirotetramat. No negative effects were reported (Temerowski, 2006).

As spirotetramat is a new active substance, there are no studies, published papers or further reports available.

Comments

Biochemical aspects

After oral administration at a dose of 2 or 100 mg/kg bw, spirotetramat was rapidly absorbed in rats. The extent of absorption in the single low-dose test was 95%. The maximum plasma concentration of radiolabel was reached 0.1–2.0 h after dosing. Concentrations of radiolabel in tissues and organs at 48 h were very low (< 0.2%). Excretion was mainly urinary and was very rapid (essentially complete within 24 h). Faecal excretion accounted for 2–11% of the administered dose in rats. No parent compound was detected in the excreta. Only very minor metabolites (< 0.7% of the administered dose) were not identified. The main metabolic reaction was cleavage of the ester group, producing the enol that is subsequently metabolized to a range of metabolites.

In male rats given a high dose of spirotetramat at 1000 mg/kg bw, it was found that only 27% of the administered dose was excreted in the urine after 24 h. In addition, concentrations of radiolabel in the plasma were slightly higher than in the liver and kidney, and the decline in concentrations of radiolabel found in the tissues was minimal from 1 h to 8 h after dosing, with considerable quantities still remaining at 24 h (approximately 25%). These findings were consistent with saturation of cellular transport mechanisms, which may result in decreased excretion via urine and faeces and a potential for the accumulation of spirotetramat metabolites in the body after repeated high doses. The results of physiologically-based pharmacokinetic simulations supported this conclusion and suggested that repeated daily doses of spirotetramat at > 500 mg/kg bw lead to non-linear elimination kinetics, resulting in a higher than expected body burden in studies with repeated doses, despite some evidence of reduced absorption at such high doses.

In a comparative study of in vitro metabolism in hepatocytes from male rats, mice, and humans, differences in the proportions of several metabolites were observed; however, spirotetramat-enol was the first and most prominent metabolite detected and accounted for 66% and 100% of all metabolites in these studies, in mice and rats respectively. The relative efficiency of enol glucuronidation in isolated hepatocytes was: mouse > human > rat.

Toxicological data

Spirotetramat has low acute toxicity: oral and dermal LD_{50}s in rats were > 2000 mg/kg bw; the inhalation LC_{50} was > 4.18 mg/l of air. Spirotetramat is not a skin irritant in rabbits, although it is an irritant to rabbit eyes. Spirotetramat exhibited a skin sensitization potential in guinea-pigs (Magnussen & Kligman test) and mice (local lymph node assay).

In general, there were no target organs or effects that were common to all species. However, it should be noted that there were indications of immune-related effects in several species.

Mice appeared to be insensitive to toxicity caused by spirotetramat. In repeat-dose studies, mice given diets containing spirotetramat at the highest dose of 5000 ppm (equal to 1415 mg/kg bw per day), 7000 ppm (equal to 1305 mg/kg bw per day) or 7000 ppm (equal to 1022 mg/kg bw per day) for 4 weeks, 14 weeks or 18 months, respectively, showed no toxicological effects.

In a 14-week dietary study of toxicity in rats, the NOAEL was 2500 ppm (equal to 148 mg/kg bw) on the basis of decreased body-weight gain, an increased incidence of abnormal spermatozoa and hypospermia, an increased incidence of tubular degeneration, decreased absolute testicular weight, and accumulation of alveolar macrophages in the lungs of rats at 10 000 ppm (equal to 616 mg/kg bw per day). However these effects were reversible within 4 weeks in most rats after cessation of treatment. In the 1-year dietary study in rats, the NOAEL was 250 ppm (equal to 13.2 mg/kg bw per day) on the basis of an increased incidence of accumulation of alveolar macrophages in the lungs of males at 3500 ppm (equal to 189 mg/kg bw per day). Effects on body weight, and testes and sperm were observed at 7500 ppm.

The thymus and the thyroid were the main target organs in dogs. Reduced weight accompanied by histological evidence of involution and atrophy of the thymus was observed at 6400 ppm in the 4-week dose range-finding study, at 4000/2500 ppm in the 13-week study, and at 600 and 1800 ppm in the 1-year study. Although there was no clear dose–response relationship in the 1-year study, these findings were considered toxicologically significant because they occurred in all studies and because there were other indications that spirotetramat interferes with the immune system (skin sensitization, effect on lungs in rats, and allergic contact dermatitis in humans). Decreases in T4 and T3 concentrations were also observed, with an overall NOAEL of 600 ppm. Changes at this dose were inconsistent. Reduced body weight and haematological effects were observed at higher doses.

The occasional brain ventricular dilatation observed at 600 ppm (one male and one female) and at 1800 ppm (one male) in the 1-year study was not accompanied by any clear histopathological alterations. In addition, brain ventricular dilatation is occasionally reported to occur spontaneously in the strain of dogs used in the test. Consequently, the Meeting considered that this finding was of uncertain toxicological significance.

The Meeting concluded that the NOAEL in the 1-year study in dogs was 200 ppm, equal to 5 mg/kg bw per day, on the basis of effects on the thymus. This NOAEL is also protective for the equivocal findings of changes in thyroid hormones, and the brain ventricular dilatation of uncertain significance seen at 600 ppm.

Spirotetramat was tested in an extensive range of studies of genotoxicity. Negative results were found in studies in vivo and in vitro, except for one weakly and equivocally positive result in a study for chromosomal aberrations in vitro that was not reproduced in a second study with higher concentrations. The Meeting concluded that spirotetramat is unlikely to be genotoxic.

The carcinogenic potential of spirotetramat was studied in mice and rats. Spirotetramat was not found to be carcinogenic in either species. In rats, the NOAEL was 250 ppm, equal to 12.5 mg/kg bw per day, on the basis of structural changes in the kidney (renal tubular dilatation) at 3500 ppm. In this study, effects on the lungs were characterized by an increased incidence of accumulation of alveolar macrophages and of interstitial pneumonia at 7500 ppm and inconsistently at lower doses. These changes were of uncertain significance, possibly being indicative of effects of spirotetramat

on the immune system. Effects on body-weight gain, the testes, epididymis and bile duct were also observed at 7500 ppm.

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

Further support for findings of testicular effects in rats given spirotetramat at a high dose was provided by the results of a dose range-finding one-generation study and a two-generation study of reproductive toxicity. In the one-generation dietary study of reproductive toxicity in rats, severe toxicity was observed in sperm (motility and malformation) of parental males at 10 000 ppm (equal to 538 mg/kg bw per day), resulting in no pregnancies with a NOAEL of 6000 ppm (equal to 320 mg/kg bw per day). However, minimal effects on sperm parameters were observed in the F_1 generation at 6000 ppm (equivalent to 400 mg/kg bw per day in parents) with a NOAEL of 500 ppm (equal to 27.8 mg/kg bw per day in parents). At this dose, a significant (–14%) decline in pup-weight gain, possibly secondary to decreases in maternal body weight was observed. In the two-generation study of reproductive toxicity, abnormal sperm cells were reported in the F_1 generation, but not in parental male rats at 6000 ppm (equal to 487 mg/kg bw per day) and decreased reproductive performance was also observed in one of these males. Offspring toxicity also included decreased body weight in F_1 and F_2 pups in both sexes during lactation at 6000 ppm (equal to 419 mg/kg bw per day). Effects observed in the parental generation were reduction of body weight and/or body-weight gain, reduced terminal body weight, reduced food consumption (females) and increased multifocal tubular dilatation in the kidneys in rats at 6000 ppm. The NOAEL for parental toxicity was 1000 ppm (equal to 70.7 mg/kg bw per day) on the basis of decreases in body-weight gain in the parental generation. The NOAEL for reproductive toxicity was 1000 ppm (equal to 79.5 mg/kg bw in F_1 males) on the basis of abnormal sperm-cell morphology in the F_1 generation. The NOAEL for offspring toxicity was 1000 ppm on the basis of growth retardation at 6000 ppm.

Two studies of developmental toxicity in rats treated by gavage have been performed. Inconsistent and equivocal effects on the offspring, including retarded ossification and increased wavy ribs, were observed in one study at doses of 140 and 20 mg/kg bw per day. Maternal effects consisting mainly of reduced body-weight gain were observed at 1000 mg/kg bw per day and were associated with reduced offspring weight, reduced fetal weight, retarded ossification and a slight increase in the frequency of fetuses with any malformations. The overall NOAEL for maternal toxicity was 140 mg/kg bw per day and the overall NOAEL for developmental toxicity was 140 mg/kg bw per day.

In a study of developmental toxicity in rabbits treated by gavage, severe maternal toxicity was observed, including death and abortion, at 160 mg/kg bw per day. No effects were observed at 40 mg/kg bw per day, except one abortion, which was considered to be incidental. No significant effects were observed in the offspring and the NOAEL was 160 mg/kg bw per day, the highest dose tested. The NOAEL for maternal toxicity was 40 mg/kg bw per day.

The effects on sperm, testes and epididymis were studied in more detail in rats given spirotetramat at a dose of 1000 mg/kg bw per day. It was observed that the decreased epididymal sperm counts occurred after 21 days and not after 10 days of treatment. In another study in rats given the enol metabolite, testicular/sperm toxicity similar to that caused by spirotetramat was observed. Thus these effects are unlikely to be due to the presence of the acyl chain of this compound.

The Meeting concluded that spirotetramat causes toxicity in the testes and sperm that, at higher doses, affects reproductive performance in rats. The NOAEL for testes and sperm effects was 169 mg/kg bw per day, with a LOAEL of 370 mg/kg bw per day in a 2-year study in adult rats, and a NOAEL of 79.5 mg/kg bw per day and a marginal LOAEL of 400 mg/kg bw per day in young rats, respectively. The Meeting observed that these effects occurred at doses higher than those causing other types of systemic toxicity, on which the acceptable daily intake (ADI) and acute reference dose (ARfD) were based.

Two studies of acute oral neurotoxicity in rats have been conducted. The overall NOAEL was 100 mg/kg bw per day on the basis of urine staining and slight declines in motor and locomotor activity in male rats at 200 mg/kg bw per day.

Studies with four metabolites found in animals and plants – spirotetramat-*cis*-ketohydroxy, spirotetramat-desmethyl-ketohydroxy, spirotetramat-mono-hydroxy and spirotetramat-di-hydroxy – showed that these substances were of low acute oral toxicity in female rats (LD_{50} > 2000 mg/kg bw) and not mutagenic in an assay for gene mutation in strains of *S. typhimurium*. The plant-specific metabolite spirotetramat-enol-glucoside is rapidly absorbed from the gastrointestinal tract and extensively metabolized and excreted within 24 h. The metabolites formed from this compound in rats do not differ from those found in the metabolism study with spirotetramat in rats.

Spirotetramat caused two proven cases of allergic contact dermatitis in workers handling undiluted active ingredient. Neither a questionnaire survey among staff exposed to spirotetramat nor yearly surveillance of 12 workers exposed to spirotetramat revealed any further cases of sensitization.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.05 mg/kg bw per day based on a NOAEL of 200 ppm (equal to 5 mg/kg bw per day) identified on the basis of thymus involution in a 1-year study in dogs and with a safety factor of 100.

The Meeting established an ARfD of 1 mg/kg bw, based on a NOAEL of 100 mg/kg bw identified on the basis of altered motor and locomotor activity and FOB changes in a single-dose study in rats treated by gavage and with a safety factor of 100. This ARfD provides adequate protection from maternal toxicity and abortion observed at 160 mg/kg bw per day in the study of developmental toxicity in rabbit, even in the unlikely event that the observed effect could be attributed to a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of carcinogenicity [a]	Toxicity and carcinogenicity	7000 ppm, equal to 1022 mg/kg bw per day[c]	—
Rat	Two-year study of carcinogenicity [a]	Toxicity	250 ppm, equal to 12.5 mg/kg bw per day	—
		Carcinogenicity	7500 ppm, equal to 373 mg/kg bw per day [c]	3500 ppm, equal to 169 mg/kg bw per day
	Multigeneration reproductive toxicity [a d]	Parental	1000 ppm, equal to 70.7 mg/kg bw per day	6000 ppm, equal to 419 mg/kg bw per day
		Offspring	1000 ppm, equal to 79.5 mg/kg bw per day	6000 ppm equivalent to 400 mg/kg bw per day
		Reproductive	1000 ppm, equal to 79.5 mg/kg bw per day	6000 ppm, equal to 486.7 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	140 mg/kg bw per day	1000 mg/kg bw per day
		Embryo and fetal toxicity	140 mg/kg bw per day	1000 mg/kg bw per day
	Acute oral neurotoxicity[b,d]		100 mg/kg bw (overall)	200 mg/kg bw

Species	Study	Effect	NOAEL	LOAEL
Rabbit	Developmental toxicity [b]	Maternal toxicity	40 mg/kg bw per day	160 mg/kg bw per day
		Embryo and fetal toxicity	160 mg/kg bw per day[c]	—
Dog	1-year study of toxicity [a]	Toxicity	200 ppm, equal to 5 mg/kg bw per day	600 ppm, equal to 19 mg/kg bw per day

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

Estimate of acceptable daily intake for humans

0–0.05 mg/kg bw

Estimate of acute reference dose

1 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 exposure

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

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid and nearly complete absorption,
Distribution	Extensive, highest in liver and kidney
Potential for accumulation	No evidence of significant accumulation at low doses
Rate and extent of excretion	Very fast and almost complete within 48 h.
Metabolism in animals	Extensive. Main metabolite spirotetramat-enol was formed by cleavage of ester bond. Other minor metabolites are formed by oxidative transformation or conjugation.
Toxicologically significant compounds (animals, plants and environment)	Spirotetramat and spirotetramat -enol

Acute toxicity

Rat, LD50, oral	> 2000 mg/kg bw
Rat, LD50, dermal	> 2000 mg/kg bw
Rat, LC50, inhalation	> 4.18 mg/l air (nose only)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Irritating
Skin sensitization	Skin sensitization potential (Magnussen & Kligman test) in guinea-pigs and local lymph node assay in mice

Short-term studies of toxicity

Target/critical effect	Thymus involution
Lowest relevant oral NOAEL	200 ppm (equal to 5 mg/kg bw per day) 1-year study in dogs
Lowest relevant dermal NOAEL	> 1000 mg/kg bw per day
Lowest relevant inhalation NOAEL	No data

Genotoxicity

	No genotoxic potential

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Kidney (tubular dilatation), decreased absolute weight
Lowest relevant NOAEL	2-year, rat, 250 ppm (equal to 12.5 mg/kg bw per day)
Carcinogenicity	No carcinogenic potential in mice and rat

Reproductive toxicity

Reproduction target/critical effect	Abnormal sperm in F1 at parentally toxic dose
Lowest relevant reproductive NOAEL	Parental toxicity: 1000 ppm (equal to 70.7 mg/kg bw per day) Offspring toxicity: 1000 ppm (equal to 79.5 mg/kg bw per day) Reproductive toxicity: 1000 ppm (equal to 79.5 mg/kg bw per day)
Developmental target/critical effect	Increased incidence of retarded ossification in fetuses at maternally toxic doses in rats. None in rabbits.
Lowest relevant developmental NOAEL	Maternal toxicity: 40 mg/kg bw per day (rabbit) Developmental toxicity: 140 mg/kg bw per day (rat)

Neurotoxicity/delayed neurotoxicity

Acute neurotoxicity	On the basis of behavioural effects, NOAEL was 100 mg/kg bw per day in rats

Medical data

	Two proven cases of allergic contact dermatitis in workers handling undiluted active ingredient. No other effects were observed.

Summary

	Value	Study	Safety factor
ADI	0–0.05 mg/kg bw	Dog, 1-year study of oral toxicity	100
ARfD	1 mg/kg bw	Rat, studies of acute oral neurotoxicity	100

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TRIAZOLE FUNGICIDE METABOLITES

(1,2,4-TRIAZOLE; TRIAZOLE ALANINE; TRIAZOLE ACETIC ACID)

First draft prepared by
P.V. Shah[1] and Maria Tasheva[2]

[1] United States Environmental Protection Agency, Office of Pesticide Programs, Washington, DC, USA; and
[2] National Service for Plant Protection, Ministry of Agriculture and Food, Sofia, Bulgaria

Explanation

1,2,4-Triazole, triazole alanine, triazole acetic acid, triazole pyruvic acid and triazole lactic acid are the common metabolites derived from triazole-containing fungicides that act by inhibiting sterol synthesis. The levels of triazole pyruvic acid and triazole lactic acid found in metabolism studies are low, and no toxicological data on these compounds were available, therefore, they were not considered by the present Meeting.

1,2,4-Triazole, triazole alanine and triazole acetic acid are the commonly used names for IUPAC nomenclatures 1*H*-1,2,4-triazole (CAS No. 288-88-01), 1,2,4-triazolyl-3-alanine (CAS No. 10109-05-4), and 1*H*-1,2,4-triazol-l-ylacetic acid (CAS No. 28711-29-7), respectively. These three metabolites commonly occur as plant or soil metabolites and are collectively known as the "triazole derivative metabolites". Triazole alanine and triazole acetic acid residues are primarily associated with plant commodities, while 1,2,4-triazole is mainly associated with animal commodities, lesser amounts of this compound being found in plant commodities. 1,2,4-Triazole is found in studies of the

metabolism of triazole fungicides in rats, where it may constitute approximately 1–65% of the dose, depending on the parent compound administered.

Triazole alanine was first evaluated by the JMPR in 1989. The Meeting concluded from the available data at that time that residues of triazole alanine arising from the use of triazole fungicides do not present a toxicological hazard. The Meeting has not previously evaluated 1,2,4-triazole and triazole acetic acid. These compounds were reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR) and following recommendations made by the JMPR in 2007 (*General consideration 2.3*). A group of manufacturers of these pesticides have formed a taskforce known as the "Triazole Derivative Metabolite Group" (TDMG) and made a joint submission of toxicological data to the JMPR. All pivotal studies with triazole alanine and triazole acetic acid were certified as complying with good laboratory practice (GLP), unless otherwise stated in the toxicological monograph.

The toxicological database for 1,2,4-triazole was sufficient for the evaluation of this compound, while the toxicological databases for triazole alanine and triazole acetic acid were more limited. The Meeting concluded that adequate studies were available to establish an acceptable daily intake (ADI) for 1,2,4-triazole and a group ADI for triazole alanine and triazole acetic acid. This decision was based on the following considerations:

— The chemical structures of triazole alanine and triazole acetic acid are closely related and the two substances have similar physicochemical characteristics.

— Both triazole alanine and triazole acetic acid have the 1,2,4-triazole active (protonated) nitrogen bonded to carbon, which significantly reduces the toxicity of triazole alanine and triazole acetic acid.

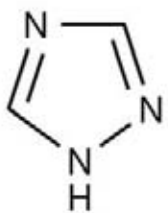
— The available toxicological data suggest that triazole alanine and triazole acetic acid are less toxic than 1,2,4-triazole.

— Triazole alanine and triazole acetic acid have similar toxicokinetic profiles in that they are rapidly eliminated, primarily in the urine and mostly as the parent compound.

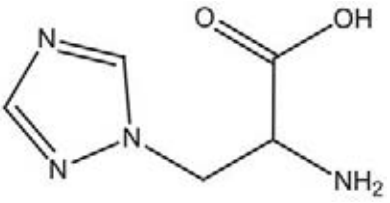
The Meeting recommended that the ADI and acute reference dose (ARfD) values established for these triazole metabolites may be used in risk assessment on a case-by-case basis, depending on the residue and toxicity profile of the parent compound. The Meeting also noted that these values may also be useful in a combined risk assessment, depending on the exposure situation, including whether exposure to these metabolites comes from more than one source of the parent conazoles.

The data for 1,2,4-triazole, triazole alanine and triazole acetic acid are described in the present monograph.

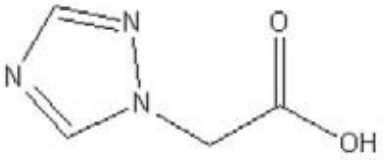
Figure 1. Chemical structures of 1,2,4-triazole, triazole alanine and triazole acetic acid



1,2,4-Triazole



Triazole alanine



Triazole acetic acid

1,2,4-TRIAZOLE

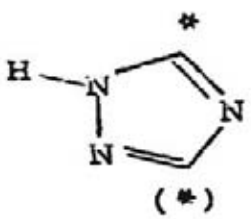
Evaluation for acceptable daily intake

Unless otherwise stated, studies evaluated in this monograph were performed by GLP-certified laboratories and complied with the relevant Organisation for Economic Cooperation and Development (OECD) and/or United States Environmental Protection Agency (US EPA) test guideline(s).

1. Biochemical aspects

1.1 Absorption, distribution, and excretion

Figure 2. Position of radiolabel on 1,2,4-triazole



* Indicates position of radiolabel.

Rats

In a pharmacokinetic study, groups of two male and two female Sprague-Dawley rats were given a single dose of [^{14}C]-labelled 1,2,4-triazole (radiolabelled at positions 3 and 5 of the triazole ring; purity, > 98%) at 0.4, 48.8, or 865.7 mg/kg bw by gavage in deionized water. Treated rats were individually housed in stainless steel metabolism cages. Urine and faeces were collected daily for 7 days, after which the rats were killed. Selected tissues and blood samples were collected and analysed for radioactivity. The study was not conducted in accordance with GLP.

Recovery of total radiolabel was nearly complete (100.2–102.8%); [^{14}C]-ring labelled 1,2,4-triazole was readily absorbed and most of the radiolabel was excreted within 24 h (Table 1). Urine was the predominant pathway of excretion. Excretion of radiolabel in the urine of male rats during 7 days was 93.5%, 80% and 87.6% at the lowest, intermediate and highest dose, respectively. In female rats, urinary excretion of radiolabel in 7 days was 90.6%, 92.4% and 91.9% at the lowest, intermediate and highest dose, respectively. Total excretion of radiolabel in the faeces during 7 days ranged from 6.5% to 19.9% (average, 10.4%) in male and female rats at the doses tested. Absorption was nearly complete based on urinary excretion during 7 days. Most of the absorption occurred within 48 h. Absorption was not saturated at the highest dose tested. The excretion pattern did not exhibit sex-related variability. Total radiolabel in tissues ranged from 0.6% to 1.6% of the administered dose at the doses tested, indicating that 1,2,4-triazole and its metabolites do not undergo significant sequestration (Lai & Simoneaux, 1986).

In a separate pharmacokinetic study, groups of male Sprague-Dawley rats were given a single dose of [^{14}C]-labelled 1,2,4-triazole (radiolabelled at positions 3 and 5 of the triazole ring; purity, 97%) in physiological saline solution. The absorption, distribution, and elimination of radiolabel were studied in groups of five rats that received a single oral dose at 1.0 mg/kg bw by stomach tube or single intravenous doses of 0.1, 1.0, 10.0 or 100 mg/kg bw, and in groups of four male Sprague-Dawley rats with cannulated bile ducts that received an intravenous or intraduodenal dose of 1.0 mg/kg bw. Urine and faeces were collected up to 48 h after dosing. Bile was collected up to 24 h after dosing. Elimination of radiolabel in expired air was also measured. Blood and a number of organs and tissues were collected for analysis from groups of five male Sprague-Dawley rats up to 6 days after a single intravenous dose of 1.0 mg/kg bw. Urine, faeces, bile, blood, tissues and expired air were analysed for radiolabel. These studies were not conducted in accordance with GLP.

Approximately 0.1% of the administered dose was detected in expired air after oral and intravenous administration in 30 h. The main route of excretion after intravenous or oral administration was the urine (92–94% after 48 h), irrespective of dose or route of administration (Table 2). Approximately 3–5% of the administered dose was recovered in the faeces within 48 h after administration by oral

Table 1. Excretion of radiolabel by rats given a single oral dose of [^{14}C]1,2,4-triazole[a]

Sample	Radiolabel (% of total radioactivity)					
	Dose (mg/kg bw)					
	0.4		48.8		865.7	
	Males	Females	Males	Females	Males	Females
Urine, day 1	81.0	78.3	65.2	69.7	43.7	46.9
Urine, day 2	10.2	10.5	11.7	19.2	36.5	38.5
Urine, days 3–7	2.3	1.8	3.1	3.5	7.4	6.5
Urine, subtotal	93.5	90.6	80.0	92.4	87.6	91.9
Faeces, day 1	7.4	6.5	17.9	9.0	3.2	5.9
Faeces, day 2	1.0	0.6	1.5	0.9	2.0	2.8
Faeces, days 3–7	0.3	0.3	0.5	0.5	1.3	0.5
Faeces, subtotal	8.7	7.4	19.9	10.4	6.5	9.2
Tissue residues	0.8	0.6	0.8	0.9	1.6	1.3
Cage wash	0.0	0.5	0.3	0.8	1.0	1.2
Total recovery	103.0	99.1	101.0	104.5	96.7	103.6

From Lai & Simoneaux (1986)

[a] Group size, $n = 2$.

and intravenous routes. Approximately 2% of the administered dose was recovered in the gastrointestinal tract at 48 h. Based on urinary and faecal excretion, the oral absorption and excretion was very rapid. The bioavailability of the oral dose was virtually 100%. In bile duct-cannulated rats, approximately 12% of the administered dose was recovered in the bile at 24 h after intravenous or intraduodenal administration. About 60–65% of the administered dose was eliminated via the urine, and 3.5–4% via the faeces. Approximately 14–18% of the administered dose was recovered in tissues and 6–9% in the gastrointestinal tract of the bile duct-cannulated rats within 24 h. Thirty minutes after an intravenous dose of 1 mg/kg bw, almost 100% of the administered dose was detectable in the body, while the body minus gastrointestinal tract contained about 90% of the administered dose. The concentration of radiolabel in the body declined to 55% of the administered dose at 8 h after intravenous administration, and to about 1.9% of the administered dose at 3 days. The radiolabel was largely uniformly distributed in the rat's body: the highest concentrations at 30 min after administration were measured in muscle and lung (1.2 µg/g), the lowest in renal fat (0.48 µg/g). The decline of radiolabel in plasma and in most tissues was approximately monoexponential up to about day 3 after administration, with an elimination half-life of about 12 h. The concentrations of radiolabel in the body minus gastrointestinal tract and in selected tissues were very low at 6 days after administration. The observed concentrations were at or below the limits of quantitation of 2–7 ng/g. Autoradiography of rats dosed at 1 mg/kg bw via intravenous administration indicates that the radioactivity was detectable in all tissues and organs with the exception of compact body tissues at 5 min (Weber et al., 1978).

1.2 Metabolism

In a separate study of metabolism, 10 male Sprague-Dawley rats were given [^{14}C]-labelled 1,2,4-triazole (radiolabelled at position 3 and 5 of the triazole ring; purity, > 99%) as a single dose at 10 mg/kg bw by gavage in physiological saline solution (0.9% sodium chloride). Urine samples were collected at 0–8 h and 8–24 h. Only urine samples were collected for identification of metabolites since previous studies have shown that greater than 90% of the orally administered dose of 1,2,4-triazole was excreted in the urine in 24 h. Urinary metabolites were separated by three different solvent systems using thin-layer chromatography (TLC). The TLC separations were visualized by extinction of fluorescence induced by ultraviolet (UV) light (250 nm), development of autoradiograms, and a TLC scanner. Radioactivity of the samples was determined by means of liquid scintillation spectrometry. The verification of 1,2,4-triazole, the major urinary elimination product, was done by "reverse isotope dilution analysis" in which 500 mg of unlabelled 1,2,4-triazole was added to a 10 ml sample of urine

Table 2. Excretion of radiolabel by male rats at 48 h after a single oral or intravenous dose of [^{14}C]1,2,4-triazole

Sample	Radiolabel (% of total radioactivity)				
	Dose (mg/kg bw)				
	Intravenous				Oral
	0.1	1	10	100	1
Urine	93.9	92.6	92.1	93.9	91.9
Faeces	3.9	5.0	5.0	3.6	5.4
Total elimination	97.8	97.6	97.1	97.5	97.3
Tissue residues	1.7	2.1	2.4	2.0	2.2
Gastrointestinal tract	0.51	0.44	0.51	0.47	0.47

From Weber et al. (1978)

[a] Group size, n = 5.

(collected at 0–8 h) and specific radioactivity was checked after further clean-up steps (12 ethyl acetate extractions and three recrystallizations). This study was not conducted in accordance with GLP.

The TLC chromatographs showed only one major zone (95.3%) representing the parent compound. There were three other zones representing less than 2.8% of the urinary radioactivity. The absence of a change in specific radioactivity in the reverse isotope dilution analysis confirmed the identity of the elimination product (Ecker, 1980).

2. Toxicological studies

(a) Acute toxicity

The acute toxicity of 1,2,4-triazole is summarized in Table 3.

Rats

Groups of three male Crl:CD BD rats were given a single dose of 1,2,4-triazole (purity, 92.8%) at 500 or 5000 mg/kg bw by gavage in 0.5% methyl cellulose. Treated rats were subjected to gross necropsy at the end of a 14-day observation period. Body weights were recorded at initiation and at the end of the study. All rats at 5000 mg/kg bw died within 10 min after dosing. No treatment-related clinical signs were observed at 500 or 5000 mg/kg bw. Gross necropsy of decedents revealed reddened duodenum and reddened glandular portion of the stomach. There was no mortality at 500 mg/kg bw. No treatment-related necropsy findings were observed at termination in the group at 500 mg/kg bw. There were no apparent effects on the body weights of survivors. The oral median lethal dose

Table 3. Acute toxicity of 1,2,4-triazole

Species	Strain	Sex	Route	LD50 (mg/kg bw)	LC50 (mg/l air)	Reference
Rat	Crl:CD BR	Males and females	Oral	> 500 (males) < 5000 (females)	—	Procopio & Hamilton (1992)
	Wistar II albino	Males and females		1650 (males) 1648 (females)	—	Thyssen & Kimmerle (1976)
Rat	Wistar II albino	Males and females	Dermal	4200 (males) 3129 (females)	—	Thyssen & Kimmerle (1976)
Rabbit	New Zealand White	Males and females		>200 (males) < 2000 (females)	—	Procopio & Hamilton (1992)
Rat	Wistar II albino	Male	Inhalation (4 h)	—	Exposure not demonstrated	Thyssen & Kimmerle (1976)
Mice	NMRI	Male	Inhalation (6 h)	—	Exposure not demonstrated	Thyssen & Kimmerle (1976)
Rabbits	New Zealand White	Male	Dermal irritation	Slightly irritating	—	Procopio & Hamilton (1992)
Rabbits	New Zealand White	Not reported	Dermal irritation	Not irritating	—	Thyssen & Kimmerle (1976)
Rabbits	New Zealand White	Male	Ocular irritation	Severely irritating	—	Procopio & Hamilton (1992)
Rabbits	New Zealand White	Not reported	Ocular irritation	Severely irritating	—	Thyssen & Kimmerle (1976)
Guinea-pig	Crl:(HA)BRDunkin Hartley	Male	Dermal sensitization (maximization test)	Not sensitizing	—	Frosch (1998)

(LD_{50}) in rats was greater than 500 mg/kg bw but less than 5000 mg/kg bw (Procopio & Hamilton, 1992).

In a second study, groups of 15 male and 15 female Wistar II albino rats were given a single dose of 1,2,4-triazole (purity, technical grade) at 100, 250, 500, 1000, 1250, 1500, 1750, 1850, 2000 or 2500 mg/kg bw by gavage. The test material was emulsified in distilled water and Cremophor EL in a volume of 10 ml/kg bw. Treated rats were observed for 14 days. Treated rats were subjected to gross necropsy at the end of a 14-day observation period.

Mortality was observed at doses of 1250 mg/kg bw or higher (1 h to 12 days after dosing). The following clinical signs were observed: sedation, breathing disorders, reduction in general well-being, lying in abdominal or side position (at higher doses). The symptoms appeared within an hour of administration and were observed for a maximum of up to 13 days after dosing. No treatment-related necropsy findings were observed at termination. The oral LD_{50} of 1,2,4-triazole in rats was 1650 and 1648 mg/kg bw for males and females, respectively (Thyssen & Kimmerle, 1976).

(b) Dermal administration

Rats

1,2,4-Triazole (purity, technical grade) at doses of 1000, 2000, 2500, 3500, 4000 or 5000 mg/kg bw was applied to the shaved skin of groups of 5–20 male and 5–20 female Wistar-II rats. The test substance was moistened with Cremophor EL. The application site was covered by occlusive dressing for 24 h then washed with water and soap. Treated rats were observed for 14 days.

Mortality (within 1–9 days) was observed at doses of 2500 mg/kg bw or higher. The observed clinical signs were similar to those in the previously described study of acute oral toxicity (i.e. sedation, breathing disorders, reduction in general well-being, abdominal or side position). The dermal LD_{50} of 1,2,4-triazole in rats was 4200 and 3129 mg/kg bw for males and females, respectively (Thyssen & Kimmerle, 1976).

Rabbits

Groups of two male New Zealand White rabbits were given 1,2,4-triazole (purity, 92.8%) at a dose of 200, 2000 or 5000 mg/kg bw in 0.9% saline as a paste applied to the shaved skin. Each application site was covered with an impervious cuff for 24 h. The cuff was removed and the application site was wiped with a paper towel. The rabbits were observed for 14 days. Body weights were recorded at initiation and at the end of the study. All rabbits were subjected to a post-mortem examination at termination. Skin irritation was evaluated according the method of Draize on days 1 to 14.

At 2000 and 5000 mg/kg bw, all treated rabbits died by day 4. The following clinical signs were observed at 2000 and 5000 mg/kg bw: abdominal breathing, ataxia, clear nasal discharge, gasping, iritis, moribundity, salivation, scant droppings, soft faeces, tremors, and yellow nasal discharge. These signs were observed in 1 day and lasted for 3 days. Slight to moderate skin irritation was observed. No deaths occurred at 200 mg/kg bw, no clinical signs were observed, and gross necropsy revealed no visible lesions. There were no apparent body-weight effects in the survivors. Erythema and slight oedema of the skin at the application site were observed during the study. The dermal LD_{50} in rabbits was greater than 200 mg/kg bw but less than 2000 mg/kg bw (Procopio & Hamilton, 1992).

(c) Inhalation

Mice and rats

In a study of acute toxicity after inhalation, groups of five male Wistar II albino rats and 10 male NMRI mice were exposed (whole-body, in a 10 l inhalation chamber) to 1,2,4-triazole (purity,

technical grade) for 4 h or 6 h, respectively. Air was passed at 2 l/min through the test material contained in a dust tower. No substance vaporized or atomized in the 4-h and 6-h experiments. Rats and mice tolerated the inhalation periods without signs of toxicity. No irritant effect on the mucous membrane of the eyes and noses of the animals was observed. The inhalation median lethal concentration (LC_{50}) in mice and rats could not be determined because exposure to the test substance was not demonstrated (Thyssen & Kimmerle, 1976).

(d) Dermal irritation

In a study of primary dermal irritation, two male New Zealand White rabbits were exposed dermally to 0.5 g of 1,2,4-triazole (purity, 92.8%) moistened with 0.9% saline (1 : 1 w/v) and applied on two patches of shaved skin for 24 h. One site was intact and other site was abraded. The treated area was covered with an impervious cuff for 24 h. The application site was wiped clean with a paper towel after 24 h. Skin irritation was scored according to the method of Draize at 24 h, 72 h and 7 days after patch removal. For the intact skin, one application site exhibited moderate erythema at 24 h. For the abraded skin, very slight erythema was observed on two application sites at 24 h and on one application site at 72 h. The primary irritation score (average of values for 24 h and 72 h) was 0.25 for the intact skins and 0.38 for the abraded skin. It was concluded that 1,2,4-triazole was slightly irritating to the skin of rabbits (Procopio & Hamilton, 1992).

In a second study of primary dermal irritation, two New Zealand White rabbits (sex not reported) were exposed dermally to 500 mg of 1,2,4-triazole (purity, technical grade), applied under cellulose patches for 24 h to the hairless skin of the ears using an adhesive dressing. No sign of skin irritation was observed after removal of the dressing or during the 7-day post-treatment observation period. Under the study conditions, 1,2,4-triazole was not irritating to the skin of rabbits (Thyssen & Kimmerle, 1976).

(e) Ocular irritation

In a study of primary ocular irritation, 0.1 g of 1,2,4-triazole (purity, 92.8%), was instilled into the conjunctival sac of one eye of two male New Zealand White rabbits. Irritation was scored by the Draize method at 4, 24, 48, 72 and 96 h and at 7 and 14 days. Corneal, iridal and conjunctival effects were observed at 4 h. Corneal and conjunctival effects were no longer evident on day 7; iridal effects were no longer evident on day 14. 1,2,4-Triazole was considered to be severely irritating (ocular effects were reversible within 21 days but not within 7 days) to the eyes of rabbits (Procopio & Hamilton, 1992).

In a second study of primary ocular irritation, 50 mg of 1,2,4-triazole (purity, technical grade), was instilled into the conjunctival sac of one eye of two New Zealand White rabbits (sex not reported). One h after application, intense reddening and a very intense swelling of the conjunctivae of the treated eyes had developed, which persisted up to 5 days after application in one animal. The conjunctivae of both animals were normal 7 days after application. During the first and second day after application, a slight, dispersed, diffuse opacity of the cornea was observed. The iris was slightly reddened and swollen. Under the study conditions, 1,2,4-triazole was severely irritating to the eyes of rabbits (Thyssen & Kimmerle, 1976).

(f) Dermal sensitization

In a study of dermal sensitization using the maximization method of Magnusson and Kligman, groups of young male Dunkin-Hartley guinea-pigs (5 in the control group, and 10 in the treatment

group) were given 1,2,4-triazole (purity, > 98%) at a concentration of 10% (in water) for intradermal induction, 75% (in Vaseline) for topical induction, and 75% (in Vaseline) for the challenge. The positive-control group was treated with benzocaine under the same experimental conditions.

The test substance caused slight skin irritation with and without Freund complete adjuvant after intradermal injection. No skin reaction was recorded after dermal induction. At 48 h and 72 h after the start of epidermal challenge (i.e. 24 h and 48 h after removal of the dressings) no signs of allergic skin reactions were noted in the test or control groups. The positive controls gave positive responses at 48 h and 72 h after the start of epidermal challenge. Under the study conditions, 1,2,4-triazole was not a skin sensitizer in guinea-pigs as determined by the maximization method (Frosch, 1998).

2.1 Short-term studies of toxicity

Mice

In a 28-day repeat study of oral toxicity, groups of 15 male and 15 female CD-1 {[ICR]/BR} mice were given diets containing 1,2,4-triazole (purity, 99.9%) at a concentration of 0, 50, 250, 500 or 2000 ppm (equal to 0, 9, 47, 90 and 356 mg/kg bw per day for males and 0, 12, 60, 120, and 479 mg/kg bw per day for females). Diets were prepared weekly and stored at room temperature. The stability, homogeneity and dietary concentrations were confirmed analytically. Treated mice were observed at least daily for signs of toxicity and mortality. Detailed clinical examinations were performed weekly. Body weight and food consumption were measured weekly. Blood samples were collected at termination for haematology and clinical chemistry measurements. All mice were subjected to gross pathological examination. Selected organs were weighed. Selected tissues from the mice in the control group and from mice at the highest dose were collected for histopathological examination.

Diets were stable for 7 days at room temperature. The test article homogeneity results were within the acceptable range. The test substance concentration analysis indicated that the measured test concentrations ranged between 96–99% of the target concentration.

No treatment-related effects were observed on survival, clinical signs, body weight, food consumption, haematological or clinical chemistry parameters, organ weights, or on grossly observable lesions. Liver-enzyme analyses were not reported for this study owing to improper storage of the liver samples. Purkinje cell loss, which was noted in a 90-day study in rats (Wahle & Sheets, 2004) and in mice (Wahle, 2004), was not found in any of the cerebellar brain sections from males in the control group or males at 2000 ppm in this study. The only treatment-related effects found included slight

Table 4. Incidence of testicular/epididymal effects in male mice given diets containing 1,2,4-triazole for 28 days

Effect[a]	Dietary concentration (ppm) [b]				
	0	50	250	500	2000
Testicular degeneration	3	ND	ND	ND	5
Apoptotic bodies (testes)	2 (1.0)[a]	4 (1.0)	1 (1.0)	3 (1.0)	5 (1.0)
Spermatid degeneration/depletion/asynchrony (testes)	1 (1.0)	1 (1.0)	1 (1.0)	0	5 (1.4)
Focal tubular atrophy (testes)	1 (1.0)	2 (1.0)	1 (2.0)	2 (2.0)	4 (1.8)
Exfoliated germ cells/debris (epididymides)	0	1 (1.0)	1 (3.0)	0	3 (2.0)

From Wahle (2004a)
ND, not deermined.
[a]Average severity score of lesion is given in parentheses: 1 (minimal) to 5 (severe).
[b] For all groups, $n = 15$

testicular degeneration in 5 out of 15 male mice at 2000 ppm, which was accompanied by apoptotic bodies within the lumen of spermatogenic tubules in stages I–VII (5 out of 15 mice), minimal to slight spermatid degeneration/depletion/asynchrony (5 out of 15 mice), focal tubular atrophy (4 out of 15 mice), and a slight increase in the incidence of exfoliated germ cells/debris in the epididymides (3 out of 15 mice) (Table 4).

The lowest-observed-adverse-effect level (LOAEL) for male CD-1 mice treated with 1,2,4-triazole for 28 days was 2000 ppm (equal to 356 mg/kg bw per day) on the basis of slight testicular effects. A LOAEL for female CD-1 mice was not identified.

The no-observed-adverse-effect level (NOAEL) was 90 mg/kg bw per day and 479 mg/kg bw per day, for male and female CD-1 mice respectively (Wahle, 2004a).

Mice

In a 90-day study of oral toxicity, groups of 20 male and 20 female CD-1 ([ICR]/BR) mice were given diets containing 1,2,4-triazole (purity, 99.9%) at a concentration of 0, 500, 1000, 3000 or 6000 ppm (equal to 0, 80, 161, 487, or 988 mg/kg bw per day for males and 0, 105, 215, 663, or 1346 mg/kg bw per day for females). An additional group of 15 males and 15 females were given diets containing 1,2,4-triazole at a concentration of 0, 3000, or 6000 ppm for 28 days and then killed for hepatic-enzyme analyses. Diets were prepared every 2 weeks and stored at freezer temperature until use. The stability, homogeneity and dietary concentrations were confirmed analytically. Treated mice were observed at least daily for signs of toxicity and mortality. Detailed clinical examination was performed weekly. Body weight and food consumption were measured weekly. Blood samples were collected at 4 weeks and at termination for haematology and clinical chemistry measurements. In addition, activities of selected hepatic enzymes were measured in mice in the control group and in mice at 3000 and 6000 ppm at 4 weeks, and in mice in the control group and mice at 6000 ppm at week 13. All mice were subjected to gross pathological examination. Selected organs were weighed. Selected tissues from mice in the control group and mice at the highest dose were collected for histopathological examination.

Diets were stable for 35 days stored at freezer temperature. The results for test-article homogeneity were within the acceptable range (< 10%). The analysis of test substance concentration indicated that the measured test concentrations ranged between 94–95% of the target concentration.

There were no treatment-related effects on mortality. Treatment-related clinical signs included tremors in males and females at 3000 and 6000 ppm, yellow staining of the ventrum in males at 3000 and 6000 ppm, food spillage in females at 3000 and 6000 ppm, and rough coat in males at 6000 ppm. The tremors were first observed on day 30 of treatment in males and day 35 in females. Male body weights were consistently decreased throughout the study in the groups at 3000 and 6000 ppm (94% and 84% of values for controls, respectively), but the decreases seen at 3000 ppm were not considered to be toxicologically significant. A consistent decrease in food consumption was seen only in the males at 6000 ppm. In females, a treatment-related decrease in body weight was seen throughout the study in the group at 6000 ppm (91% of values for controls), but no significant change in food consumption was noted for this, or any other, treated female group.

No toxicologically relevant changes in haematological and clinical chemistry parameters were observed. Total cytochrome P450 activity was increased in males and females at 6000 ppm compared with controls. In liver tissue, increased activities of 7-ethoxycoumarin deethylase (ECOD), 7-ethoxyresorufin deethylase (EROD), and aldrin epoxide (ALD) were seen after 4 weeks exposure to 1,2,4-triazole at 3000 or 6000 ppm or to 13 weeks exposure to 1,2,4-triazole at 6000 ppm in males and females. These changes in enzyme activities were not correlated with changes in liver weights or histopathology, and were therefore considered to be adaptive changes. There was also a marginal increase in UDP-glucuronyltransferase (GLU-T) activity. Increased activity of GLU-T was observed in males and females compared with controls after 28 days and 90 days at 6000 ppm.

Gross lesions attributable to exposure to 1,2,4-triazole were limited to males at 6000 ppm and included an increased incidence of rough coat and wet/stained ventrum.

Absolute brain weights were reduced in the males at 3000 and 6000 ppm (95% and 91% of control values, respectively) and the females at 6000 ppm (93% of control values; Table 5). This effect was seen in conjunction with decreased numbers of Purkinje cells in the cerebella of groups of males (15 out of 20) and females (10 out of 18) at 6000 ppm (Table 6). These changes were described as involving cell-body loss and, in some cases, degeneration of axons in the white matter of the cerebellar folia. Of males at 6000 ppm, 9 out of 11 mice showing tremors also had Purkinje cell loss; of females at the highest dose, 1 out of 3 of the mice with tremors had Purkinje cell loss.

Absolute testes weights were significantly decreased in males at 6000 ppm (87% of control values) and non-significantly decreased in the groups at 3000 and 1000 ppm (92% each of control values). In conjunction with this decrease in testicular weights, histopathological changes in the testes were observed, including increased incidence of apoptotic-like bodies (4 out of 20, 4 out of 20, 7 out of 20, 11 out of 20, and 12 out of 20 of the males in the control group and the males at 500, 1000, 3000, and 6000 ppm, respectively); spermatid degeneration, depletion, and asynchrony (5 out of 20 and 15 out of 20 of the males at 3000 and 6000 ppm, respectively), and minimal or slight focal tubular atrophy (2 out of 20, 3 out of 20, and 10 out of 20 of the males at 1000, 3000, and 6000 ppm, respectively). A correlated change in the epididymides of the group at 6000 ppm was considered secondary to the testicular effects and consisted of increased germ cells and debris in the luminal duct (10 out of 20) and one male with aspermia. Minimally increased apoptotic-like bodies and tubular atrophy seen at 1000 ppm were considered treatment-related but not adverse. Testicular atrophy at 1000 ppm was considered to be spontaneous due to their limited tissue distribution (focal and/or unilateral) and the lack of accompanying spermatid degeneration/depletion/asynchrony.

Table 5. Mean absolute and relative organ weights of mice fed diets containing 1,2,4-triazole for 90 days

Parameter	Dietary concentration (ppm)				
	0	500	1000	3000	6000
Males (n = 20 per group)					
Terminal body weight (g)	37.3 ± 2.2	337.0 ± 2.2	36.4 ± 2.9	34.9* ± 2.0	31.3* ± 1.7
Absolute brain weight (g)	0.488 ± 0.023	0.491 ± 0.023	0.476 ± 0.025	0.465* ± 0.024 (95)[a]	0.445* ± 0.019 (91)
Relative brain/body (%)	1.328	1.378	1.365	1.376	1.462*
Absolute testes weight (g)	0.253 ± 0.044	0.247 ± 0.038	0.233 ± 0.024 (92)	0.233 ± 0.044 (92)	0.219* ± 0.029 (87)
Relative testes/body (%)	0.688 ± 0.131	0.692 ± 0.110	0.669 ± 0.081	0.687 ± 0.121	0.719 ± 0.103
Females (n = 19–20 per group) [b]					
Terminal body weight (g)	29.1 ± 2.4	28.4 ± 1.8	28.4 ± 2.1	28.7 ± 3.1	26.6* ± 1.6 (93)
Brain weight (g)	0.485 ± 0.031	0.489 ± 0.018	0.483 ± 0.026	0.475 ± 0.016	0.451* ± 0.025 (93)
Relative brain/body (%)	1.737	1.756	1.731	1.717	1.734

From Wahle (2004b)

[a] Value in parentheses is the percentage of the control value.

[b] n = 19 for females at 1000 and 3000 ppm; for all other groups of females, n = 20.

* Statistically significantly different ($p \leq 0.05$) from the controls.

The LOAEL was 3000 ppm, equal to 487 mg/kg bw per day for males, on the basis of tremors, decreased brain weight, decreased testicular weight and histopathological changes in the testes. The LOAEL was 6000 ppm equal to 1346 mg/kg bw per day for female mice, on the basis of decreased body weight, decreased body-weight gain, decreased brain weight and histopathological findings in the brain (cerebellum).

The NOAEL was 1000 ppm for males and 3000 ppm for females, equal to 161 and 663 mg/kg bw per day, respectively(Wahle, 2004b).

Rats

In a 90-day study of oral toxicity, groups of 15 male and 15 female Wistar rats were given diets containing 1,2,4-triazole (purity, 99.6%) at a concentration of 0, 100, 500, or 2500 ppm (equivalent to 0, 7.8, 37.9, or 212.3 mg/kg bw per day for males and 0, 10.2, 54.2, or 266.7 mg/kg bw per day for females). Dietary concentrations were not measured analytically. Treated rats were observed at least daily for signs of toxicity and mortality. Body weights were measured weekly. Blood samples

Table 6. Incidence of selected histopathology findings in mice given diets containing 1,2,4-triazole for 90 days

Organ/description of finding[a]	Dietary concentration (ppm)				
	0	500	1000	3000	6000
Males (n = 20 per group)					
Testes:					
No abnormality	15	16	12	9	1
Apoptotic-like bodies	4 (1.0)	4 (1.3)	7 (1.1)	11* (1.3)	12** (1.2)
Spermatid degeneration/depletion/asynchrony	1 (1.0)	0	0	5 (1.4)	15** (2.0)
Tubular atrophy	0	0	2 (1.5)	3 (1.0)	10** (1.8)
Epididymides:					
Germ cells and debris in duct lumen	0	0	0	0	10** (2.5)
Aspermia, relative or absolute	0	0	0	0	1 (3.0)
Brain:					
Purkinje cell loss	0	0	0	0	15** (1.7)
Gliosis	0	0	0	0	0
Eye:					
Retinal degeneration	0	0 [b]	0 [c]	0 [c]	2 (2.0)
Erosion	0	0 [b]	0 [c]	1 (3.0) * [b]	2 (1.5)
Females (n = 20 per group)					
Brain:					
Purkinje cell loss	0	0	0	0	10** [c] (1.3)
Gliosis	0	0	0	0	1 (2.0)
Eye:					
Retinal degeneration	0	0 [b]	0 [b]	0 [b]	0
Erosion	0	3 (3.0) * [b]	0 [b]	0 [b]	0

From Wahle (2004b).

[a] Values in parentheses are mean severity, ranging from 1 (minimal) to 5 (severe).

[b] $n = 1$ for males at 500, 1000 and 3000 ppm, n = 4 for females at 500 ppm and n = 2 for females at 1000 and 3000 ppm.

[c] $n = 18$ owing to loss of cerebella of two females at the highest dose.

* Statistically significantly different ($p \leq 0.05$) from the controls, calculated by reviewer.

** Statistically significantly different ($p \leq 0.01$) from the controls, calculated by reviewer.

were collected from five males and five females per group at termination for haematology and clinical chemistry measurements. Rectal temperatures of all rats were measured at 1 month and at termination. Urine analysis was conducted at termination. All rats were subjected to gross pathological examination. Selected organs were weighed. Selected tissues from five males and five females per group were collected for histopathological examination. This study was not conducted in accordance with GLP regulations.

No mortality was observed during the study. Two males and two females at 2500 ppm exhibited temporary slight convulsions. Rectal body temperature was not affected by treatment. Food intake was reduced at 2500 ppm during the first 2 weeks of treatment, but in overall terms food intake was similar in all groups. Body-weight gains were reduced at 2500 ppm, leading to total weight-gain deficits of 12% and 8% for males and females, respectively, relative to values for untreated controls. There were statistically significant changes in erythrocyte parameters after 1 and 3 months in males at 2500 ppm that pointed to slight microcytic hypochromic anaemia. No other alterations in haematological parameters were observed in females at 1 month or at termination. Clinical chemistry demonstrated no deviations of toxicological significance. Urine analysis did not reveal any differences between controls and treated groups at 1 month or at termination. There were no significant differences in protein-bound-iodine (PBI) measurement between controls and treated groups at 1 month or at termination. There were deviations in absolute organ weights at 2500 ppm, particularly in males, that were attributed to lower terminal body weights. Gross findings at necropsy were not considered to be associated with treatment. Histopathological evaluation revealed slight to moderate fat accumulation in liver parenchymal cells in three males at 2500 ppm.

The NOAEL was 500 ppm, equivalent to 37.9 and 54.2 mg/kg bw per day in males and females, respectively, on the basis of retarded body-weight development, temporary slight effects on the central nervous system (CNS), decreases in erythrocyte parameters (males only) and hepatocellular fat accumulation (males only) at 2500 ppm (equivalent to 212.3 and 266.7 mg/kg bw per day for males and females, respectively (Bomhard et al., 1979).

In a combined short-term study of toxicity and neurotoxicity, groups of 20 male and 20 female Wistar (Crl:WI[Glx/BRL/Han]IGS BR) rats were given diets containing 1,2,4-triazole (purity, 99.9%) at a nominal concentration of 0, 250, 500, 3000 or 1000/4000 ppm (1000 ppm for the first 4 weeks and 4000 ppm, thereafter) for approximately 14 weeks. The mean daily intake was 16, 33, 183 and 210 mg/kg bw per day for males and 19, 41, 234 and 275 mg/kg bw per day for females at 250, 500, 3000 and 1000/4000 ppm, respectively. Diets were prepared weekly and stored at room temperature. The stability, homogeneity and dietary concentrations were confirmed analytically. Treated rats were observed at least daily for signs of toxicity and mortality. Detailed clinical examinations were performed weekly. Body weight and food consumption were measured weekly. Ophthalmoscopic examination was performed on all rats before the study and all survivors at termination. Blood samples were collected from 10 males and 10 females per group at termination for haematology and clinical chemistry measurements. Urine samples were collected for urine analysis at termination. Neurobehavioural assessment (functional observational battery [FOB] and motor activity testing) was performed on 12 males and 12 females per group before exposure and during weeks 2, 4, 8 and 13. At study termination, 10 males and 10 females per group were killed and perfused in situ for neuropathological examination. Of the perfused rats, all rats in the control group and those at the highest dose were subjected to histopathological evaluation of brain and tissues of the peripheral nervous system. The remaining 10 males and 10 females per group were killed and standard tissues were weighed and examined for gross and microscopic pathology. In addition, the liver was snap-frozen and analysed for metabolizing enzymes.

Diets were stable for 35 days stored at room temperature. The results for test-article homogeneity were within the acceptable range (< 10%). The analysis of test-substance concentration indicated that the measured test concentrations ranged between 94% and 98% of the target concentrations.

Tremors were observed in one female from the group at 1000/4000 ppm. No other treatment-related clinical signs of toxicity were observed in any rat during exposure, or during daily and weekly examinations. All rats survived to scheduled termination. Beginning on approximately day 42, body weight was significantly or slightly decreased in both sexes at 1000/4000 ppm (92–96% of control value) and at 3000 ppm (93–96% of control value). Final body weight was 7% and 6% lower than values for controls in males and females at 3000 ppm, respectively; while reductions compared to control of 8% and 5% were noted at 1000/4000 ppm in males and females, respectively. Overall (days 0–91), body-weight gain was significantly decreased in males and females (79% of control value) at 1000/4000 ppm and in males (82% of control value) and females (81% of control value) at 3000 ppm. Food consumption in treated rats was comparable to that of the control group for most treated rats, with increased consumption in males and females at the highest dose during the latter half of the exposure period.

Evaluation of haematological parameters provided no treatment-related adverse findings in both sexes at termination. A slight decrease in serum triglyceride and uric acid was observed in males at 3000 ppm and 1000/4000 ppm. A dose-related decrease in thyroid stimulating hormone (TSH) was seen in males at all doses, statistically significant at 500 ppm and above (74–65% of control values). No treatment-related effects were observed in concentrations of thyroxin (T4) and triiodothyronine (T3) in males. A slight decrease in TSH was seen in female rats, but a dose–response relationship was not evident. In the absence of any thyroid histopathology and changes in T3 and T4 concentrations, and overall susceptibility of rat thyroid to chemical perturbation, these decreases in TSH were not considered to be toxicologically relevant.

No treatment-related effects were observed on urine analysis parameters in both sexes. Ophthalmoscopic examinations at termination revealed retinal degeneration in 4 out of 20 males and 2 out of 20 females at 3000 ppm, in 5 out of 20 males and females at 1000/4000 ppm compared with 2 out of 20 males in the control group and no females in the control group. There was a slight increase in numbers of corpora lutea in females at 3000 ppm and 1000/4000 ppm; although not statistically significant, this is consistent with similar findings in a study of reproductive toxicity (Wahle & Sheets, 2004). No other treatment-related effects were observed on gross or microscopic examination at necropsy.

Absolute brain weight was significantly decreased in males and females at 3000 ppm (93–95% of control value), significantly decreased in males at 1000/4000 ppm (94% of control value) and non-significantly decreased in females at 1000/4000 ppm (94% of control value) (Table 7). Analysis

Table 7. Organ changes in in rats fed diets containing 1,2,4-triazole for 14 weeks

Parameter	Dietary concentration (ppm)				
	0	250	500	3000	1000/4000
Males					
Absolute brain weight (g)	2.05	2.02	2.01	1.94*	1.92*
Relative brain weight (%)	0.48	0.48	0.47	0.49	0.50
Females					
Absolute brain weight (g)	1.91	1.89	1.88	1.78*	1.81
Relative brain weight (%)	0.83	0.78	0.81	0.79	0.79
Total corpora lutea	33 ± 9b	NE	33 ± 6	41 ± 9	40 ± 14
Recently cycling corpora lutea	16 ± 5	NE	17 ± 4	21 ± 4	19 ± 6

From TDMG (2008) and Wahle & Sheets (2004).
NE, not evaluated.
[a] $n = 10$.
[b] ± standard deviation

Table 8. Incidence of histopathological findings in brain and peripheral nerves in rats given diets containing 1,2,4-triazole for 14 weeks

Finding [a]	Dietary concentration (ppm)			
	0	500	3000	1000/4000
Males				
Tissues examined	10	10	10	10
Brain, level 7 (cerebellum):				
Degeneration/necrosis	—	—	10* (2.5)	9* (2.8)
Degeneration, nerve fibre	1 (1.0)	2 (1.0)	4 (1.3)	5 (1.0)
Ganglion, dorsal root:				
Vacuolization	1 (1.0)	3 (1.0)	9* (1.1)	7* (1.0)
Chromatolysis	5 (1.0)	3 (1.0)	10* (1.2)	10* (1.0)
Degeneration, nerve fibre	2 (1.5)	5 (1.0)	9* (1.3)	10* (1.1)
Nerve, sural, left: Degeneration, nerve fibre	—	2 (1.0)	6* (1.0)	9* (1.1)
Nerve, sural, right: Degeneration, nerve fibre	—	2 (1.0)	7* (1.1)	8* (1.4)
Nerve, sciatic, left: Degeneration, nerve fibre	2 (1.0)	2 (1.0)	9* (1.3)	9* (1.2)
Nerve, sciatic, right:				
Degeneration, nerve fibre	2 (1.0)	3 (1.0)	9* (1.0)	9* (1.4)
Nerve, tibial, left: Degeneration, nerve fibre	4 (1.0)	5 (1.0)	10* (1.5)	10* (1.5)
Nerve, tibial, right:				
Degeneration, neuronal	—	—	—	1 (1.0)
Degeneration, nerve fibre	2 (1.0)	1 (1.0)	8* (1.5)	10* (1.4)
Females				
Tissues examined	10	10	10	10
Brain, level 7 (cerebellum):				
Degeneration/necrosis	—	—	10* (2.6)	10* (2.3)
Degeneration, nerve fibre	1 (1.0)	—	1 (1.0)	1 (1.0)
Ganglion, dorsal root				
Vacuolization	1 (1.0)	—	—	2 (1.0)
Chromatolysis	3 (1.0)	4 (1.0)	10* (1.0)	10* (1.0)
Degeneration, nerve fibre	8 (1.1)	—	2 (1.3)	5 (1.1)
Nerve, sural, left: Degeneration, nerve fibre	1 (1.0)	1 (1.0)	5 (1.4)	8* (1.4)
Nerve, sural, right: Degeneration, nerve fibre	1 (1.0)	1 (1.0)	4 (1.3)	3 (1.7)
Nerve, sciatic, left: Degeneration, nerve fibre	5 (1.0)	5 (1.0)	7 (1.0)	9 (1.1)
Nerve, sciatic, right: Degeneration, nerve fibre	7 (1.0)	4 (1.0)	6 (1.2)	7 (1.1)
Nerve, tibial, left: Degeneration, nerve fibre	4 (1.0)	3 (1.3)	7 (1.3)	10* (1.4)
Nerve, tibial, right:				
Degeneration, neuronal	—	—	—	—
Degeneration, nerve fibre	3 (1.0)	1 (1.0)	6 (1.0)	7 (1.6)

From TDMG (2008) and Wahle & Sheets, 2004).

—, zero incidence.

[a] The average severity of the lesion, graded from 1 (minimal) to 5 (severe), is shown in parentheses.

* $p < 0.05$.

of the activity of selected hepatic enzymes indicated slightly increased activities in males and females at 3000 and 1000/4000 ppm.

The neurotoxicity part of the study included neurobehavioural (FOB and motor activity) and neuropathology assessments. In the FOB, effects were observed in males and females at 1000/4000 and 3000 ppm with the incidence and severity increased at week 8. Males were more severely affected than females. The effects, which were not observed during pre-treatment testing or in rats in the control group, included ungroomed appearance, red nasal and lacrimal stain, yellow-stained urine, muscle fasciculations, tremors, gait incoordination, decreased activity in the open field, decreased rearing, uncoordinated righting reflex and increased foot-splay. A decrease in motor and locomotor activity was also observed in males at 3000 ppm, during week 4 only. At necropsy, terminal body weight in the neurotoxicity groups was non-significantly decreased in males and females at 1000/4000 (93–94% of control value) and 3000 ppm (94% of control value). Fixed absolute brain weight was significantly decreased in males and females at 1000/4000 ppm (95% of control value) and non-significantly decreased in both sexes at 3000 ppm (96% of control value). No treatment-related effects were observed on macroscopic examination. On microscopic examination, nerve-fibre degeneration was observed in multiple peripheral nerves (sciatic, tibial, sural), in the Gasserian and dorsal root ganglia and in the spinal nerve roots with increased incidence and severity in males and females at 1000/4000 and 3000 ppm as compared with the control group. Males were more severely affected than females. In the brain, lesions were found in the more anterior dorsal cerebellum (level 7) in males and females at 1000/4000 ppm and 3000 ppm. The lesions included mineralization, axonal degeneration, degeneration/necrosis and nerve-fibre degeneration in males but were limited to degeneration/necrosis and nerve-fibre degeneration in females. In females at 1000/4000 ppm, an increase in nerve-fibre degeneration was also reported in brain levels 4 and 5.

The LOAEL for toxicity/neurotoxicity was 3000 ppm, equal to 183 mg/kg bw per day, on the basis of decreased body weight and body-weight gain, FOB changes, decreased absolute brain weight, and increased incidence of neuropathology findings in the peripheral and central nervous system. The NOAEL was 500 ppm, equal to 33 mg/kg bw per day (Wahle & Sheets, 2004).

2.2 Long-term studies of toxicity and carcinogenicity

No studies were submitted.

2.3 Genotoxicity

1,2,4-Triazole has been tested for genotoxicity in a battery of studies in vitro. Two assays for reverse mutation in bacteria, a test for mutation at the *Hgprt* locus and for chromosomal aberration

Table 9. Results of studies of genotoxicity in vitro with 1,2,4-triazole

End-point	Test object	Concentration	Purity (%)	Result	Reference
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	10–5000 µg/ plate ± S9 in water	99.7	Negative[a]	Poth (1989)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537;	100–7500 µg/ plate ± S9 in water	92.8	Negative	Melly & Lohse (1982)
Hgprt forward mutation	Chinese hamster ovary cells	43.2–691 µg/ml ± S9	99.3	Negative	Schisler & Kleinert (2007a)
Chromosomal aberration	Rat lymphocytes	10.8–691 µg/ml ± S9	99.3	Negative	Schisler & Kleinert (2007b)

S9, 9000 × *g* supernatant from rodent liver.
[a] Toxic effects at 1000 and 5000 µg/plate.

gave negative responses in the presence or absence of metabolic activation. No studies on germ cells were available. The results of studies of genotoxicity with 1,2,4-triazole are summarized in Table 9.

2.4 Reproductive toxicity

(a) Multigeneration study

In a two-generation study of reproduction, groups of 30 male and 30 female Wistar Hannover rats were given diets containing 1,2,4-triazole (purity 99.9–101%) at a concentration of 0, 250, 500 or 3000 ppm. For the F_0 and F_1 dams, concentrations of the test article in the diet were reduced to 0, 139/104, 278/207 and 1666/1245 ppm during days 0–7 and 7–21 of lactation, respectively, to maintain a constant intake of the test substance. One litter was produced in each generation; insufficient F_1 pups from the group at the highest dose were produced from which to select parental animals and this dose group was cancelled. Pre-mating doses for the F_0 parental animals in the control group and groups at the lowest dose, intermediate dose and highest dose were 0, 15.4, 30.9, and 188.6 mg/kg bw per day, respectively, for males and 0, 17.5, 36.2, and 217.9 mg/kg bw per day, respectively, for females. Pre-mating doses for the treated F_1 parental rats in the control group and groups at the lowest, and intermediate dose were 0, 16.0, and 32.0 mg/kg bw per day, respectively, for males and 0, 18.9, and 37.5 mg/kg bw per day, respectively, for females. F_0 and F_1 parental rats were given test or control diet for 10 weeks before mating, throughout mating, gestation, and lactation, and until sacrifice.

In addition to the normal end-points for reproductive toxicity, the brains of F_0 adult rats in the control group, and at the intermediate and highest dose, and F_1 adults (non-perfused) in the control group and at the intermediate dose and F_1 and F_2 weanlings (perfused) in the control group and at the intermediate dose were evaluated histopathologically, including morphometric measurement of weanling brains. Stability, homogeneity and dietary concentrations were confirmed analytically. Rats were observed for clinical signs and monitored for changes in body weight and food consumption. All rats placed on study underwent a post-mortem examination, which included documenting and saving all gross lesions, weighing designated organs and collecting representative tissue specimens for histopathological evaluation and sperm analysis.

1,2,4-Triazole was stable at room temperature for 7 days. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage (88.5–105.2%) was acceptable.

No treatment-related deaths or clinical signs of toxicity were observed in any group during the study. Absolute body weight of the F_0 males and females at the highest dose was significantly ($p \leq 0.05$ or 0.01) less than that of controls beginning at days 42 and 21, respectively, and continuing throughout premating (males, 92–95% of control values; females, 95–97% of control values). The most pronounced effect was reduced body-weight gain during the first 2 weeks of premating; during this time, males and females gained 65% and 20%, respectively, of the control value. Overall premating-weight gain was 72% and 63% of controls for males and females at the highest dose, respectively. Absolute body weight and body-weight gain by the F_0 rats at the lowest and intermediate dose were similar to those of controls throughout the pre-mating interval. Absolute body weight was significantly less ($p \leq 0.05$ or 0.01) than that of the controls for the F_1 males at the intermediate dose throughout premating and for the F_1 males at the lowest dose beginning on day 14 of the pre-mating period (Table 10). Absolute body weights and body-weight gains for the treated F_1 females were similar to those of the control group during premating. Food consumption, calculated as g/kg per day, was increased in all treatment groups for most of the treatment period; this increase was statistically significant for F_0 females and for F_1 adults at multiple time-points during the pre-mating treatment period.

The results of gross necropsy were unremarkable. F_0 males and females at the highest dose had significantly reduced terminal body weight and absolute brain weight compared with those of

the controls; several other organs also showed a decrease in weight at the highest dose, including the thyroid in both sexes and spleen in females. In the F_1 adults, males at the lowest and intermediate dose had a significantly reduced final body weight and males at the intermediate dose had decreased absolute brain weight. Absolute spleen weight decreased in F_1 females, statistically significantly at

Table 10. Mean body weight, body-weight gain and food consumption in F_1 adults during the pre-mating interval in a two-generation study in rats fed diets containing 1,2,4-triazole

End-point [a]	Dietary concentration (ppm)		
	0	250	500
Males			
Body weight (g):			
Day 0	266.2 ± 3.98	254.3 ± 4.01	250.6* ± 3.96 (94)[b]
Day 14	328.6 ± 4.54	314.2* ± 4.32 (96)	307.1** ± 4.99 (93)
Day 28	372.3 ± 5.40	350.8** ± 4.64 (94)	342.1** ± 5.55 (92)
Day 42	399.4 ± 6.08	377.8* ± 5.22 (95)	367.3** ± 6.05 (92)
Day 70 (end of premating)	437.7 ± 8.24	418.0 ± 5.90 (95)	405.4** ± 6.65 (93)
Day 98 (termination)	461.9 ± 7.18	435.2* ± 7.32 (94)	428.4** ± 6.89 (93)
Body-weight gain (g):			
Days 0–14[c]	62.4	59.9 (96)	56.5 (90)
Premating[c]	171.5	163.7 (95)	154.8 (90)
After pairing[c]	24.2	17.2	23
Food consumption (g/kg per day):			
Days 0–7	91.0 ± 1.21	92.8 ± 0.96	93.4 ± 1.15
Days 28–35	60.7 ± 0.68	62.2 ± 0.52	63.2* ± 0.67
Days 49–56	52.5 ± 1.54	56.8* ± 0.70	56.8* ± 0.55
Days 63–70	51.7 ± 0.69	53.2 ± 0.83	54.1 ± 0.68
Females			
Body weight (g):			
Day 0	172.3 ± 2.44	166.7 ± 1.82	169.1 ± 2.72
Day 14	193.4 ± 2.39	189.0 ± 2.07	192.0 ± 3.00
Day 28	211.1 ± 2.67	204.4 ± 2.17	205.9 ± 3.28
Day 42	222.0 ± 2.61	214.2 ± 2.33	218.7 ± 3.64
Day 70 (end of premating)	236.2 ± 3.07	227.5 ± 2.64	230.8 ± 3.66
Body-weight gain (g):			
Days 0–14[c]	21.1	22.3	22.9
Premating[c]	63.9	60.8	61.7
Food consumption (g/kg per day):::			
Days 0–7	93.7 ± 1.24	97.7 ± 1.52	98.0 ± 1.46
Days 28–35	73.0 ± 0.86	78.1** ± 1.33	77.0* ± 1.13
Days 49–56	68.6 ± 0.75	69.9 ± 1.27	68.2 ± 1.18
Days 63–70	63.8 ± 0.67	68.9** ± 1.39	69.9** ± 1.20

From Young & Sheets (2006)

[a] ± standard error

[b] Percentage of value for control group, calculated by reviewer, is given in parentheses.

[c] Calculated by reviewer from group mean values.

*$p \leq 0.05$; **$p \leq 0.01$.

both the lowest and intermediate dose. Microscopically in the F_0 rats at the highest dose, mild to moderate degeneration/necrosis was observed in the cerebellum of 30 out of 30 males and 28 out of 30 females. The average severity of the lesion (on a scale of 1–5) was 2.8 and 2.1 for males and females, respectively. Ventricular dilatation was found in one F_1 male adult at the intermediate dose.

The NOAEL for parental systemic toxicity was < 250 ppm, < 16 mg/kg bw per day, and the x LOAEL for parental systemic toxicity was ≤ 250 ppm (≤ 16 mg/kg bw per day) on the basis of decreased body weight and body-weight gain in F_1 males and decreased spleen weight in F_1 females.

No F_1 offspring at the highest dose survived lactation. For litters of both generations at the lowest and intermediate dose, live birth, viability, and lactation indices, mean litter sizes, and sex ratios were similar between the treated and control groups. No treatment-related clinical signs of toxicity

Table 11. Combined findings for male and females in a two-generation study in rats fed diets containing 1,2,4-triazole

Finding	Dietary concentration (ppm)			
	Historical control range	Current study control	250	500
F1 generation				
Pup weight (g):				
Day 0	5.4–6.1	6.2	5.9	6.1
Day 14	24.9–32.6	33.5	32.4	32.1
Day 21	39.3–48.9	50.7	49.1	48.4
Gain (0—21 days)	33.4–42.8	44.5	43.3	42.3
Preputial separation (days):	40.9–44.0	40.7	41.2	41.3
Brain weight (g):				
Absolute	1.378–1.481	1.483	1.463	1.458
Relative	—	2.947	3.047	2.956
Spleen weight (g):				
Absolute	0.209–0.240	0.236	0.236	0.227
Relative	—	0.466	0.479	0.456
F2 generation				
Pup weight (g):				
Day 0	5.4–6.1	6.21[a]	5.8**	5.7**
Day 14	24.9–32.6	32.5	30.8	31.7
Day 21	39.3–48.9	50.2[a]	46.8**	47.6*
Gain	33.4–42.8	44.0[a]	41.0**	41.8**
Preputial separation (days):	40.9–44.0	40.7[a]	41.8*	41.5
Brain weight (g):				
Absolute	1.378–1.481	1.497[a]	1.450*	1.445*
Relative	—	3.006	3.126	3.027
Spleen weight (g):				
Absolute	0.209–0.240	0.246[a]	0.215**	0.221**
Relative	—	0.492	0.46	0.461

From Young & Sheets (2006)

* $p < 0.05$; ** $p < 0.01$ (ANOVA, Dunnett)

[a] Concurrent control results outside range of historical control values.

were observed in the pups during lactation, and gross necropsy was unremarkable. Body weight and body-weight gain of the F_1 pups in the groups at the lowest and intermediate dose were similar to those of the controls throughout lactation (Table 11). F_2 male and female pups from litters at the lowest and intermediate dose had significantly lower ($p \leq 0.05$ or 0.01) body weight at birth and on day 21 of lactation compared with the controls (Table 12). Body-weight gain by the pups in both treated groups was significantly less than that of the controls during days 14–21 of lactation. For the F_1 pups, absolute and relative organ weights were similar between the treated and control groups. For F_2 pups in the groups at the lowest and intermediate dose (sexes combined) absolute weight of the brain and spleen was significantly less ($p \leq 0.05$ or 0.01) than that of the controls; in F_2 female pups, spleen weight was also statistically significantly decreased at both doses. These changes in body weights and organ-weight changes were minor and were toxicologically significant but within the higher bound of the range for historical controls. During the neuropathology assessment, no effects were seen on qualitative neuropathology, and there were no treatment-related differences in brain morphometric measurements between the treated and control groups of either generation.

The NOAEL for developmental/systemic toxicity in offspring was 500 ppm, the highest dose tested (30.9 mg/kg bw per day). No LOAEL for developmental/systemic toxicity in offspring was identified.

The fertility index was significantly reduced ($p \leq 0.01$) in F_0 rats at the highest dose compared with the controls (7.1% vs 76.7% for the controls). Only two litters containing one female pup each were produced by the F_0 dams at the highest dose. These two dams at the highest dose were the only females in the group with implantations and both dams and their pups were sacrificed before weaning. The mean number of implantations was 1.5 per dam for the females at the highest dose with litters, compared with 11.2 and 12.4 per dam for the other treated and control groups. Other end-points of reproductive performance were not affected by treatment in the F_0 generation. No treatment-related differences in mating, fertility, or gestation indices, number of days to mating, or duration of gestation were seen between the treated and control groups of the F_1 parental rats during litter production. Body weight, body-weight change, and food consumption by the F_0 and F_1 dams at the lowest and intermediate dose were similar to those of the control group during gestation and lactation.

No treatment-related differences in the in-life evaluations of the estrous cycle or estrous-cycle length were observed between the treated and control females of either generation; there was an apparent shift in the staging of estrus at sacrifice in F_1 females only. No treatment-related differences in sperm motility were found between the treated and control males of either generation. F_0 males at

Table 12. Micropathology observations in the adult parental generation in a two-generation study in rats fed diets containing 1,2,4-triazole

Observation	Dietary concentration (ppm)							
	Males				Females			
	0	250	500	3000	0	250	500	3000
Brain – tissues examined:	30	0	30	30*	30	6	30	30
Degeneration/necrosis	—	—	—	30 * (2.8)	—	—	—	28 * (2.1)
Ovaries – tissues examined:	—	—	—	—	7	5	5	10
Total corpora lutea count	—	—	—	—	24.9	23.0	15.6	41.3*
Uterus - tissues examined:	—	—	—	—	30	6	5	30
Dilatation	—	—	—	—	4 (2.3)	2 (2.5)	2 (2.0)	14* (1.8)

From Young & Sheets (2006)

[a] Average severity of lesions, graded 1 (minimal) to 5 (severe), is shown in parentheses.

* $p < 0.05$.

the highest dose had a significantly lower ($p \leq 0.05$; 74% of control) epididymal sperm count compared with the controls (Table 13). F_0 males at the intermediate and highest dose had a significantly ($p \leq 0.05$) lower percentage of normal (95.7–97% vs 98.7% for controls) sperm with concomitant increases in the percentage of abnormal (1.4–1.5% vs 0.8% for controls) and detached sperm (1.6–2.8% vs 0.5% for controls). F_1 males at the intermediate dose had slightly fewer epididymal and testicular sperm numbers and a slightly greater percentage of abnormal sperm than did the control group, but statistical significance was not attained.

F_0 females at the highest dose had significantly increased left and right ovarian weights, and an increase in the number of corpora lutea. The total numbers of corpora lutea for F_0 females in the control group and groups at the lowest, intermediate and and highest dose were, respectively, 24.9 ± 7.1, 23.0 ± 6.8, 15.6 ± 8.3, and 41.3 ± 6.5 ($p \leq 0.05$, see Table 14). For F_1 females, the total number of corpora lutea per female was significantly decreased at the intermediate dose (total numbers of corpora lutea in the control group and group at the intermediate dose were 48.9 ± 7.9 and 39.3 ± 7.4 [$p \leq 0.05$], respectively); females at the lowest dose were not evaluated. Dilatation of the uterus was seen in 14 out of 30 F_0 females at the highest dose with a severity of 1.8 compared with 4 out of 30 rats in the control group with a severity of 2.3. F_0 rats at the intermediate dose were not examined but the incidence of dilatation of the uterus was not increased in the F_1 females at the intermediate dose. There was also delay in vaginal opening in females of both generations, which was statistically significant for F_1 females at both doses ($p \leq 0.05$ or 0.01). Anogenital distance was not affected by treatment, for either sex, in F_2 pups.

The NOAEL for reproductive toxicity was 250 ppm (15.4–16 and 17.5–18.9 mg/kg bw per day for males and females, respectively) and the LOAEL for reproductive toxicity was 500 ppm (30.9–32 and 36.2–37.5 mg/kg bw per day for males and females, respectively) on the basis of an increase in abnormal sperm in F_0 and F_1 males and decreases in corpora lutea count in F_1 females.

Table 13. Sperm parameters in a two-generation study in rats fed diets containing 1,2,4-triazole[1]

Parameter	Dietary concentration (ppm)			
	0	250	500	3000
Parental F_0 males				
Motility (%)	76.2	78.9	78.9	78.9
Progressive (%)	55.9	56.5	56.4	57.3
Epididymis sperm count	58.2	57.0	65.7	43.2* (74)
Testis sperm count	72.0	63.1* (88)	64.4 (89)	61.2* (85)
Normal (%)	98.7	98.1	97.0*	95.7*
Abnormal (%)	0.8	1.0	1.4*	1.5*
Detached (%)	0.5	0.8	1.6*	2.8*
F_1 males				
Motility (%)	87.1	87.8	89.5	—
Progressive (%)	63.9	65.7	67.6	—
Epididymis sperm count	49.2	—	48.6	—
Testis sperm count	69.2	—	68.3	—
Normal (%)	98.1	—	97.9	—
Abnormal (%)	1.1	—	1.4	—
Detached (%)	0.8	—	0.7	—

From Young & Sheets (2006)

[a] Values are means; standard deviations were not given. n = 27–30 per group.

* $p \leq 0.05$.

The NOAEL for paternal toxicity was < 250 ppm (equivalent to < 16.0 mg/kg bw per day) on the basis of retarded body-weight gain at 250 and 500 ppm in F_1 males. The NOAEL for maternal toxicity was 500 ppm (equivalent to 36.2 mg/kg bw per day) on the basis of lower body weights, degenerative findings in the cerebellum, increased number of corpora lutea, and uterine horn dilatation at 3000 ppm in parental females.

The study author identified the NOAEL for reproductive toxicity was 500 ppm (equivalent to 34.4 mg/kg bw per day) on the basis of reduced fertility and decreased implantation sites at 3000 ppm.. This value was different than the NOAEL for reproductive toxicity was 250 ppm (15.4–16 and 17.5–18.9 mg/kg bw per day for males and females, respectively) established by the Meeting.

The NOAEL for developmental toxicity was > 500 ppm (equivalent to > 35.8 mg/kg bw per day) on the basis of lack of treatment-related effects in F_1 and F_2 pups at 250 and 500 ppm (Young & Sheets, 2006).

(b) Developmental toxicity

Rats

In a non-guideline, non-GLP study, 10 pregnant rats (Alpk: AP [Wistar-derived]) were given 1,2,4-triazole (purity not reported, vehicle not reported) at a dose of 0, 25 or 100 mg/kg bw per day during days 7–17 of gestation using the Chernoff-Kavlock assay. Maternal observations were restricted to body weights on days 1, 7–17, and 22. Offspring observations: litter weights of live pups on postnatal days 1 and 5, and the number of live and dead pups on these days. No specific examination for malformation was conducted.

Under the study conditions, 1,2,4-triazole had no effect on maternal weight gain, the number of viable litters, litter size, survival or postnatal-weight gain. 1,2,4-Triazole was not teratogenic in rats as determined by a modified Chernoff-Kavlock assay (Wickramaratne, 1987).

In a non-guideline, non GLP, study conducted in vitro, 1,2,4-triazole (purity not reported; vehicle, ethanol; together with flusilazole and fluconazole) was evaluated for malformations. Rat embryos (Crl:CD), aged 9.5 days (one to three somites), were exposed to the test substance at concentrations of 500 to 5000 µmol/l in vitro. After 48 h in culture, the embryos were examined morphologically using a dissecting microscope. The visceral yolk-sac diameter and the crown–rump and head length were measured, the somite number recorded, and the developmental degree evaluated according to the scoring method described by Brown & Fabio. After examination of abnormalities, a few embryos were fixed in 4% buffered formaldehyde and processed for histological examination. The remaining embryos were evaluated for total protein content and DNA content.

A significant reduction in visceral yolk-sac diameter, crown–rump length, somite number, and total score was found in embryos at 5000 µmol/l. No effects on embryonic DNA and protein content were observed. The study authors concluded that only slight developmental retardation and blood discoloration were observed with 1,2,4-triazole at the highest concentration, suggesting no teratogenic activity (Menegola et al., 2001).

In a study of developmental toxicity, groups of 25 pregnant female rats [Bor:WISW (SPF Cpb)] were given 1,2,4-triazole (purity, 95.3%) at a dose of 0, 10, 30 or 100 mg/kg bw per day by gavage in aqueous 0.5% (w/w) Cremophor EL on days 6 to 15 of gestation. Stability and concentrations were confirmed analytically. Treated rats were observed daily for clinical signs and mortality. Body weights were measured on days 0, 6–15, and 20. On day 20 of gestation, all rats underwent caesarian section. Observations included number of nidations, number of fetuses (live and dead), sex of surviving fetuses, weight of each fetus, average fetal weight per litter, runts, total and average placental weight per litter, examination of all fetuses for external malformations, investigation of a number

of fetuses (approximately 30% of total) for visceral malformations (modified Wilson technique), remaining fetuses assigned to skeletal and soft tissue evaluations.

The test substance was stable for 8 days. The analytical data indicated that the variance between nominal and actual dosage (within 10%) was acceptable.

No mortality was observed. No treatment-related clinical signs were observed. Mean body-weight gain was statistically significantly reduced at 100 mg/kg bw (79.8 g vs 92.9 g for the controls). There were no treatment-related effects on pregnancy parameters. There were no treatment-related effects on fetuses at doses of up to 30 mg/kg bw per day. A significantly lower fetal weight and simultaneously greater number of runts were observed at 100 mg/kg bw per day (Table 15). The observed malformations at 100 mg/kg bw per day affected only one fetus each and were considered to be spontaneous in nature (Table 16).

The NOAEL for maternal toxicity was 30 mg/kg bw per day on the basis of decrease in body-weight gain seen at 100 mg/kg bw per day, the LOAEL. The NOAEL for developmental toxicity was 30 mg/kg bw per day on the basis of an increased incidence of runts and lower fetal weights seen at the LOAEL of 100 mg/kg bw per day (Renhof, 1988c).

Table 14. Fetal effects in a study of developmental toxicity in rats given 1,2,4-triazole by gavage

Parameter	Dose (mg/kg bw per day)			
	0	10	30	100
No. of implantations per dam	11.6	10.5	11.4	10.6
No. of males per dam	6.5	5.1 *	6.0	5.0 *
No. of females per dam	4.5	5.0	4.6	4.5
No. of males and females per dam	11.0	10.1	10.6	9.5
No. of losses per dam	0.6	0.4	0.8	1.1
Mean weight of fetuses (g)	3.58	3.59	3.53	3.25**
Mean weight of placenta (g)	0.56	0.56	0.57	0.56
No. of fetuses per litter with minor skeletal deviations	2.00	2.41	2.84	2.42
No. of fetuses per litter with malformations	0.05	0.05	0.05	0.17
No. of runts per litter	0.33	0.23	0.53	2.21**

From Renhof (1988c) and TDMG (2008).
* $p \leq 0.05$. * * $p \leq 0.01$

Table 15. Fetal malformations in a study of developmental toxicity in rats given 1,2,4-triazole by gavage

Type of malformation	Dose (mg/kg bw per day)			
	0	10	30	100
Microphthalmia, bilateral	1	0	0	0
Microphthalmia, right side	0	1	0	1
Microphthalmia, left side	0	0	0	1
False posture of right hind leg	0	0	1	0
Anophthalmia	0	0	0	1
Dysplasia and asymmetry of body of vertebrae and vertebral arches of thoracic spine and abnormal position of one rib	0	0	0	1

From Renhof (1988c) and TDMG (2008).

In a second study of developmental toxicity, groups of 25 pregnant female rats [Bor:WISW (SPF Cpb)] were given 1,2,4-triazole (purity, 94%) at a dose of 0, 100 or 200 mg/kg bw per day by gavage in aqueous 0.5% (w/w) Cremophor EL on days 6 to 15 of gestation. Stability and concentrations of the test substance in the diet were confirmed analytically. Treated rats were observed daily for clinical signs and mortality. Body weights were measured on days 0, 6–15, and 20. Food consumption was determined from days 0–6, 6–11, 11–16, and 16–20. On day 20 of gestation, all rats underwent caesarian section. Observation included number of nidations, number of fetuses (live and dead), sex of surviving fetuses, weight of each fetus, average fetal weight per litter, runts, total and average placental weight per litter, examination of all fetuses for external malformations, investigation of a number of fetuses (approximately 30% of total) for visceral malformations (modified Wilson technique), remaining fetuses assigned to skeletal and soft tissue evaluations.

The test substance was stable for 8 days. The analytical data indicated that the variance between nominal and actual dosage (within 10%) was acceptable.

No mortality was observed. No treatment-related clinical signs were observed. Mean body-weight gain was slightly (non-significantly) reduced at 100 mg/kg bw per day. Marked reduction in body-weight gain was observed at 200 mg/kg bw per day (60.4 g vs 96.9 g in the control group). Food consumption was not affected by the treatment. There were no treatment-related effects on pregnancy parameters.

Fetal weight and placental weight were reduced at 100 and 200 mg/kg bw per day. The incidence of runts was higher at 100 and 200 mg/kg bw per day (Table 16). The incidence of fetuses with minor skeletal deviations was higher at 100 mg/kg bw per day. The number of surviving fetuses per dam was reduced at 200 mg/kg bw per day. The incidence of fetuses with malformations (cleft palates and hind legs) was higher at 200 mg/kg bw per day (Table 17).

The NOAEL for maternal toxicity was < 100 mg/kg bw per day on the basis of reduced body-weight gain at 100 and 200 mg/kg bw per day. The NOAEL for developmental toxicity was < 100 mg/kg bw per day on the basis of an increased incidence of runts, lower fetal and placental weights, and a higher incidence of minor skeletal deviations at 100 mg/kg bw. Teratogenic effects (cleft palate, hind-leg malformations) were observed at 200 mg/kg bw per day (Renhof, 1988d).

Table 16. Fetal effects in a study of developmental toxicity in rats given 1,2,4-triazole by gavage

Parameter	Dose (mg/kg bw per day)		
	0	100	200
No. of corpora lutea per dam	13.6	13.9	14.2*
No. of implantations per dam	12.5	12.2	11.8
No. of males per dam	5.9	6.0	3.1**
No. of females per dam	6.1	5.9	2.4**
No. of males and females per dam	12.0	11.9	5.5**
No. of losses per dam	0.5	0.3	6.3**
Mean weight of fetuses (g)	3.55	3.06**	2.35**
Mean weight of placenta (g)	0.59	0.52*	0.49**
Fetuses per litter with minor skeletal deviations	2.67	4.32*	2.24
Fetuses per litter with malformations	0.29	0.63	0.80*
No. of runts per litter	0.24	2.84**	4.96**

From Renhof (1988d); TDMG (2008)
* $p < 0.05$; ** $p < 0.01$

Rabbits

In a study of developmental toxicity, groups of 25 timed-mated female New Zealand White [Hra:(NZW)SPF] rabbits were given 1,2,4-triazole (purity, 99.9%) at a dose of 0, 5, 15, 30 or 45 mg/kg bw per day by gavage in aqueous 0.5% (w/w) carboxymethylcellulose on days 6 to 28 of gestation. Dosing solutions were prepared weekly and stored at refrigerator temperature. Homogeneity, stability and concentrations were confirmed analytically. Treated rabbits were observed twice per day for clinical signs and mortality. Body weight and food consumption were measured daily during the dosing period and at termination. On day 29 of gestation, all surviving does were killed and necropsied, and all fetuses were weighed, examined externally, sexed internally, and subjected to a visceral examination by gross dissection. Heads from approximately half of the fetuses in each litter were examined by serial sections, and brains from the remaining fetuses were examined in situ. All fetuses were examined for skeletal alterations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage (within 10%) was acceptable.

Five females at the highest dose were killed in a moribund condition during days 16–24 of gestation after exhibiting decreased food consumption and body-weight loss beginning as early as day 7 of gestation. Gravid uterine weights were significantly reduced in the group at the highest dose. Other abnormal clinical signs at the highest dose included the following: decreased motor activity and ptosis (5 out of 5); scant, soft, and/or liquid faeces (4 out of 5); a clear perinasal substance (3 out of 5); excessive salivation (3 out of 5); hyperpnea (2 out of 5); lacrimation (1 out of 5); head tilt (1 out of 5); and feeling cold to the touch (1 out of 5). Most of these signs occurred in does that were killed in a moribund condition. One rabbit at the highest dose delivered on day 29 of gestation, before scheduled sacrifice, and this rabbit also exhibited excess salivation on day 29. Among surviving rabbits, treatment-related clinical signs were seen in four additional animals at the highest dose and included the following: clear perinasal substance from three rabbits; decreased motor activity in two rabbits; head tilt in one rabbit; and hyperpnea in one rabbit. Among survivors, there were no treatment-related effects on body weight, body-weight gain, or food consumption, and no treatment-related gross pathology was noted.

There were no total litter losses; a single dead fetus was noted in the group at 30 mg/kg bw per day. The pre- and postimplantation losses and mean numbers of corpora lutea, implantations,

Table 17. Fetal malformations in a study of developmental toxicity in rats given 1,2,4-triazole by gavage

Type of malformation	Number of affected fetuses		
	Dose (mg/kg bw per day)		
	0	100	200
Total number of fetuses examined	253	226	138
Microphthalmia, left side	2	0	0
False posture of hind legs	0	0	1
Undescended testicle	2	11	6
Hydronephrosis	1	1	7
Multiple malformations	1	0	0
Cleft palate	0	0	4
Humeral dysplasia	0	0	1
General oedema	0	0	1
Long-bone displacia	0	0	2
Diaphragmatic hernia	0	0	1

From Renhof (1988d) and TDMG (2008).

viable fetuses, and resorptions of the treated does were similar to those of controls. There were no treatment-related effects on the fetal sex ratio. Mean fetal weight was decreased in both sexes at 45 mg/kg bw per day (males, 88% of controls, $p < 0.01$; females, 90% of controls, $p < 0.05$; Table 18). The total numbers of live fetuses (and litters) evaluated in the control group and groups at the lowest, low-intermediate, high-intermediate, and highest dose were 217 (25), 207 (24), 199 (24), 218 (25), and 157 (19), respectively. No treatment-related external or skeletal malformations/variations were observed. Treatment-related urinary tract malformations were noted in four fetuses from two litters at the highest dose. These included the following (none of which were seen in controls): low set and small kidney(s) in three fetuses from the same litter; absent left kidney and absent left ureter in one of these same three fetuses; and an absent kidney in one fetus from a different litter. No visceral malformations were seen in any other group.

The NOAEL for maternal toxicity was 30 mg/kg bw per day on the basis of mortality, decreased body weight and body-weight gains, decreased food consumption, and clinical signs (decreased motor activity, ptosis, scant, soft, and/or liquid faeces, a clear perinasal substance, excessive salivation, hyperpnea, lacrimation, head tilt, and/or feeling cold to the touch) seen at the LOAEL of 45 mg/kg bw per day. The NOAEL for developmental toxicity was 30 mg/kg bw per day on the basis of decreased fetal weight and increased incidences of urinary-tract malformations (small kidneys, absent kidney, absent ureter) seen at the LOAEL of 45 mg/kg bw per day (Hobermann, 2004).

2.5 Special studies

(a) Neurotoxicity

Neurotoxicity parameters were evaluated as a part of a short-term study of toxicity in rats (described under short-term studies of toxicity).

(b) Estrogen biosynthesis

In a non-guideline, non-GLP study conducted in vitro, 1,2,4-triazole (purity not reported) was evaluated for its action on estrogen biosynthesis. Triplicate cultures containing $0.25–0.63 \times 10^5$ viable immature rat granulosa cells were incubated in the presence of human follicle-stimulating hormone (FSH) (100 ng/ml), testosterone (10^{-7} mol/l) and the test substance (10^{-5} mol/l) for 48 h at 37 °C

Table 18. Fetal body weights in a study of developmental toxicity in rabbits given 1,2,4-triazole by gavage

Mean body weight (g/litter ± standard deviation)	Dose (mg/kg bw per day)[a]				
	0	5	15	30	45
Total fetuses	44.35 ± 3.37	43.42 ± 5.85	43.82 ± 5.70	42.48 ± 4.22	39.46 ± 5.20**
Male fetuses [a]	44.92 ± 3.78	43.91 ± 6.14	44.25 ± 5.72	42.39 ± 4.22 [24][b]	39.65 ± 4.73**
Female fetuses [a]	42.92 ± 3.95	42.79 ± 5.51 [23][c]	43.64 ± 6.17	42.40 ± 4.34	38.70 ± 5.90*

From Hobermann (2004)

[a] The number of values averaged is shown in square brackets.

[b] Litter 8081 contained no male fetuses

[c] Litter 8039 contained no female fetuses.

* $p \leq 0.05$; ** $p \leq 0.01$.

in a humidified tissue-culture incubator with 95% air/5% carbon dioxide. The medium was collected at the end of this period and stored at −20° C until analysis for estradiol and progesterone content by radioimmunoassays.

The results of the study indicated that 1,2,4-triazole had no suppressive effect on aromatase activity. The ratio of 20α-hydroxy-4-pregnen-3-one to progesterone (4 : 1 in control cultures) did not change in the presence of increasing concentrations of 1,2,4-triazole. The levels of estradiol and progesterone in rat granulosa-cell cultures were unaffected by treatment with 1,2,4-triazole. Therefore, it appeared that 1,2,4-triazole does not modulate ovarian estrogen biosynthesis in vitro (Wickings et al., 1987).

(c) Studies on metabolites

No studies on metabolites were submitted. On the basis of results of the metabolite-identification study, no studies are necessary since the major compound identified was 1,2,4-triazole itself.

3. Observations in humans

No observations in humans were submitted.

TRIAZOLE ACETIC ACID

Explanation

Triazole acetic acid (CAS No. 28711-29-7; 1,2,4-triazole-1-yl- acetic acid) is one of the three common metabolites derived from the parent triazole fungicide compounds belonging to the sterol demethylation inhibitors (the other two being 1,2,4-triazole and triazole alanine).

JMPR has not previously evaluated triazole acetic acid.

Evaluation for acceptable daily intake

Unless otherwise stated, studies evaluated in this monograph were performed by GLP-certified laboratories and complied with the relevant OECD and/or US EPA test guideline(s).

4. Biochemical aspects

4.1 Absorption, distribution, and excretion

Rats

In a pharmacokinetic study, groups of two male and female Sprague-Dawley rats were given [^{14}C]-ring labelled triazole acetic acid (purity, > 99%) as a single gavage dose at 0.58, 58.63 or 1034.69 mg/kg bw. The test substance was administered in water. Treated rats were individually housed in stainless-steel metabolism cages. Urine and faeces were collected daily for 7 days. Seven days after dosing, the rats were killed. Selected tissues and blood samples were collected and analysed for radioactivity. The study was not conducted in accordance with GLP regulations.

Total radiolabel recovery was nearly complete (100.1–107.2%). [^{14}C]-Ring labelled triazole acetic acid was readily absorbed and most of the radiolabel was excreted within 24 h. Urine was the predominant pathway of excretion. Excretion of radiolabel in the urine of male rats in 7 days was 91%; 101.7%; and 87.3% at the lowest, intermediate and highest dose, respectively. In female rats, urinary excretion of radiolabel during 7 days was 90.3%; 103.7%; and 98.7% at the lowest, intermediate, and highest dose, respectively. Total excretion of radiolabel in the faeces during 7 days ranged from 1.2% to 7.4% in male and female rats at the doses tested. Absorption was nearly complete (96.3–111.6%) on the basis of urinary excretion during 7 days. Most of the absorption occurred within 24 h. Absorption was not saturated at the highest dose tested. The excretion pattern did not exhibit sex-related variability. Total radiolabel in tissues ranged from 0.8% to 3.1% at the doses tested, indicating that triazole acetic acid and its metabolites do not undergo significant sequestration (Lai et al., 1986a).

4.2 Metabolism

A separate study of absorption and excretion was conducted to qualitatively characterize the metabolic profile of excreted triazole acetic acid. The study design and dosing was similar to the first study by Lai et al. (1986a). Urinary metabolites were identified by various TLC systems, column chromatography and GC/MS (gas chromatography/mass spectroscopy).

Most of the orally-administered dose of triazole acetic acid was rapidly eliminated in the urine within 24 h, regardless of the dose or sex. Only one single major zone with a very similar migration to the parent compound was separated by the TLC systems. After isolation and purification, derivatization and GC/MS analysis, it was concluded that the major urinary zone was unaltered parent compound. No other significant zones were detectable by autoradiography of TLC plates developed in multiple solvent systems (Lai et al., 1986b).

5. Toxicological studies

5.1 Acute toxicity

The acute toxicity of triazole acetic acid is summarized in Table 19.

Rats

Three male and three female young adult Tif:RAIf(SPF) rats were given triazole acetic acid (purity, > 99%) as a single dose at 5000 mg/kg bw by gavage in distilled water containing 0.5% carboxymethylcellulose and 0.1% polysorbate 80. Treated rats were monitored daily for mortality and clinical signs and symptoms and body weights were evaluated on days 1, 7, 14 and at death. Treated rats were subjected to gross necropsy at the end of a 14-day observation period.

No rats died during the study. Slight to moderate incidences of dyspnea, exophthalmos, ruffled fur, and hunched posture were observed after dosing. All clinical signs disappeared within 10 days after dosing. No effects on body weights were observed. No abnormalities were observed at necropsy. The LD_{50} was > 5000 mg/kg bw in male and female rats (Thevenaz, 1984).

Table 19. Acute toxicity of triazole acetic acid

Species	Strain	Sex	Route	LD50 (mg/kg bw)	Reference
Rats	Tif:RAIf(SPF)	Males and females	Gavage	> 5000	Thevenaz (1984) [a]

[a] This study was not conducted in accordance with GLP.

5.2 Short-term studies of toxicity

Rats

In a 14-day study of oral toxicity, groups of five male and five female Tif:RAIf (SPF) rats were given diets containing triazole acetic acid, (purity, 99%) at a concentration of 0, 100, 1000 or 8000 ppm, equivalent to 0, 10.6, 102.8 or 788.3 mg/kg bw per day in males and 0, 10.1, 97.2 or 703.5 mg/kg bw per day in females. Stability and dietary concentrations were confirmed analytically. Rats were inspected daily for signs of toxicity and mortality. Body weights were measured during the acclimatization period and weekly thereafter. Food and water consumption were determined weekly for each cage. Urine analysis was not performed. Ophthalmoscopic and auditory perception examinations were conducted on the control group and group at the highest dose during the acclimatization period and on day 12 of the study. At termination, blood was taken for haematological and clinical chemistry analysis. All rats sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed. Selected tissues from animals in the control group and group at the highest dose were collected for histological examination. This study was conducted in accordance with GLP.

Samples tested 50 days after preparation (stored at 22° C) were within a range of 86–99% of nominal concentrations. Test substance concentrations were 95.1, 93.1 and 89.9% of nominal, for the groups at the lowest, intermediate and highest dose, respectively. Homogeneity was not determined.

All rats survived to terminal sacrifice. It was stated that no clinical symptoms and signs of systemic toxicity were observed in the course of the study. However, no data were provided in the study report. It was stated that ophthalmic and hearing examinations did not reveal any treatment-related effects. Again, however, no data were provided in the study report. There were no treatment-related changes in body weight, food consumption, water consumption, haematology, or clinical chemistry parameters for any group of rats. Absolute liver weight in males at the highest dose was 86% of control values. The liver/body weight and liver/brain weight ratios were also decreased in males at the highest dose, to 90% and 86% of control values, respectively. A statistically-significant ($p < 0.01$) dose-related trend of decreasing liver/body weight ratios was seen in all treated groups of males. Absolute adrenal weights were 72% and 78% of control values in males at the intermediate and highest dose, respectively. Adrenal/body weight and adrenal/brain weight ratios were decreased to approximately 72% and 78% of control values in the groups at the intermediate and highest dose, respectively. Organ weights in females were comparable to those of the controls. No treatment-related gross or microscopic findings were seen in any rats.

Table 20. Results of studies of genotoxicity with triazole acetic acid in vitro

End-point	Test system	Concentration	Purity (%)	Result	Reference
Reverse mutation (Ames test)	*S. typhimurium* strains TA98, TA100, TA153 and TA1537 *E. coli* WP2P; WP2P *uvrA*	0, 20, 80, 320 1280 and 5120 µg/0.1 ml	> 99	Negative	Deparade (1984) [a]
Forward mutation	L5178Y mouse lymphoma cells	0.63, 1.25, 2.5, 5 and 10 mmol/l (±S9)	96.95	Negative	Clare (2002)
Chromosomal aberration	Human lymphocytes	2.5, 5 and 10 mmol/l (±S9)	96.95	Negative	Pitchard (2002)

S9, 9000 × *g* supernatant from rodent liver.

[a] This study was not conducted in accordance with good laboratory practice.

The NOAEL was 8000 ppm, equivalent to 703.5 mg/kg bw per day, the highest dose tested. It is difficult to interpret the data on organ weights confidently owing to the high variability and the small number of animals evaluated (Thevenaz, 1986).

5.3 Long-term studies of toxicity and carcinogenicity

No studies were submitted.

5.4 Genotoxicity

The results of studies of genotoxicity with triazole acetic acid are summarized in Table 20.

5.5 Reproductive toxicity

No studies were submitted.

5.6 Special studies

No studies were submitted.

Studies on metabolites were not submitted. On the basis of results of a metabolism-identification study, no studies are necessary since the major metabolite was triazole acetic acid itself.

6. Observations in humans

Observations in humans were not submitted.

TRIAZOLE ALANINE

Explanation

Triazole alanine (or triazolyl alanine, CAS No. 10109-05-4; 1,2,4-triazolyl-3- alanine-IUPAC; alpha-amino-1*H*-1,2,4-triazole-3-propanoic acid) is one of the three common metabolites derived from the parent triazole fungicide compounds belonging to the sterol demethylation inhibitors, the other two being 1,2,4-triazole and triazole acetic acid. It is commonly present as plant or soil metabolite.

Triazole alanine was first evaluated by the JMPR in 1989. The Meeting at that time concluded from the available data that residues of triazole alanine arising from the use of triazole fungicides do not present a toxicological hazard.

For the present evaluation, no new studies were submitted, except a pharmacokinetic study in rats.

Evaluation for acceptable daily intake

Unless otherwise stated, studies evaluated in this monograph were performed by GLP-certified laboratories and complied with the relevant OECD and/or US EPA test guideline(s).

7. Biochemical aspects

7.1 Absorption, distribution, and excretion

Rats

In a pharmacokinetic study, two groups of four male and female Tif: RAI f (SPF) rats were given [^{14}C]-labelled triazole alanine (at position 3 and 5 of the triazole ring; purity, > 99%) as a single gavage dose at 0.5 or 50 mg/kg bw. The test substance was given in water. Treated rats were individually housed in stainless-steel metabolism cages. Urine, faeces and expired air were collected at 24-h intervals for 7 days. Seven days after dosing, the rats were killed. Selected tissues and blood samples were collected and analysed for radioactivity.

Total radiolabel recovery was nearly complete (98.99–109.47%). [^{14}C]-Ring labelled triazole alanine was readily absorbed and excreted, mainly via the urine. Excretion of radiolabel in the urine of male rats in 24 h was 96.06% and 97.67% at the lowest and highest dose, respectively. In female rats, urinary excretion of radiolabel in 24 h was 92.01% and 98.96% at the lowest and highest dose, respectively. Approximately 3–7% of the administered doses were recovered in the faeces after 7 days. Less than 0.5% of the administered dose was excreted in the expired air. No tissue residues were found after 7 days at 0.5 mg/kg bw. At 50 mg/kg bw, only minute residues, not exceeding 22 ppb D,L-triazole alanine equivalents were found, mainly in the liver, kidneys and blood. The total amount remaining in the rats after 7 days did not exceed 0.01% of the administered dose. Up to 86% of the administered dose was excreted unchanged in the urine. TLC analysis (0–24 h) of the urinary metabolites revealed one major (U1) and one minor metabolite (U2), accounting for 72–86% and 8–19% of the recovered radiolabel, respectively (Hamboeck, 1983a).

In a separate non-GLP study of pharmacokinetics, groups of two male and two female Sprague-Dawley rats received radiolabeled D,L-triazole alanine (at position 3 and 5 of the triazole ring; purity, > 99%) as a single oral dose at 0.56, 54.4, or 993.7 mg/kg bw by gavage in polyethylene glycol (PEG). Urine and faeces samples were collected at 24-h intervals for 7 days. Rats were killed after 7 days and blood and various tissues were collected for analysis.

Recovery of radiolabel was nearly complete (90.7–101.2%). Urine was the main route of excretion. Within 24 h, 66.1–79.7% of the administered doses were recovered in the urine. An average of 97.4%, 87.35 and 88.2% of the total dose was excreted after 48 h at the lowest, intermediate and highest dose, respectively. Approximately 6–18% of the administered dose was recovered in the faeces after 7 days. The concentrations of radiolabel in selected tissues after 7 days were low at all doses (0.004 ppm, 0.40 ppm and 8.5 ppm at the lowest, intermediate and highest dose, respectively (Lai & Simoneaux, 1986a, 1986b).

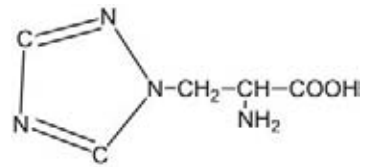
7.2 Biotransformation

In a study of metabolism identification, excreta obtained from rats given a single oral dose of [^{14}C]-D,L-triazolyl alanine at 0.5 or 50 mg/kg bw in the previously described study by Hamboeck (1983a) were used for isolation of metabolites. The excretory products in the urine and in faeces were subjected to metabolite identification using high-voltage electrophoresis (HVE), high-performance liquid chromatography (HPLC), NMR and MS analysis techniques. Approximately 69–86% of the administered dose in urine and 1–2% of the administered dose in faeces was identified as the parent compound, D,L-triazole alanine. Approximately 8–19% of the excreted dose in the

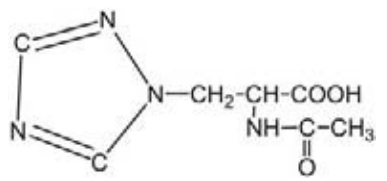
urine and < 1% of the administered dose in faeces was identified as *N*-acetyl-D,L-triazole alanine (Hamboeck, 1983b).

In a second study of metabolism identification that did not comply with GLP, excreta obtained from rats given a single oral dose of [^{14}C]-D,L-triazolyl alanine at 0.5 or 50 mg/kg bw in the previously described studies by Lai & Simoneaux (1986a, 1986b) were used for isolation of metabolites. Urine samples were subjected to TLC for metabolic identification using five different solvent systems for metabolic separations. The TLC analysis revealed that D,L-triazole alanine, represented 82–93% of the radiolabel in 0–24-h urine and *N*-acetyl-D,L-triazole alanine represented 13–30% of the radiolabel in 0–24-h urine (Lai & Simoneaux, 1986a, 1986b). Figure 3 shows the metabolic pathway of triazole alanine in rats.

Figure 3. Metabolic pathway of triazole alanine in rats



α-Amino-1,2,4-triazole-1-propanoic acid



2-Acetylamino-3-(1H-1,2,4-triazol-1-yl)-propanoic acid

Table 21. Acute toxicity with triazole alanine

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Reference
Rat	Wistar Bor:WISW (SPF-Cpb)	Males and females	Oral	> 5000	Mihail (1982)
Rat	Alderley Park (SPF)	Males and females	Oral	> 2000	Henderson & Parkinson (1980)
Mouse	NMRI (SPF-Hah)	Males and females	Oral	> 5000	Mihail (1982, 1986)

8. Toxicological studies

8.1 Acute toxicity

The acute toxicity of triazole alanine is summarized in Table 21.

(a) Lethal doses

Rats

Triazole alanine (analytically pure) was given to groups of 10 male and 10 female Bor:WISW (SPF-Cpb) Wistar rats as a single dose at 500, 1000, 2500 and 5000 mg/kg bw for fasted males and 5000 mg/kg bw for fasted females by gavage in 2.0% Cremophor EL in distilled water. A single gavage dose of 2500 and 5000 mg/kg bw was given to fed males and 5000 mg/kg bw to fed females. The rats were examined daily for clinical signs and mortality. Treated rats were subjected to gross necropsy at the end of a 14-day observation period. Body weights were recorded at initiation and at the end of the study.

Fasted male rats at 5000 mg/kg bw exhibited polyuria on the day of treatment. In some cases, the lungs were mottled, had dark discoloration, and were distended. Some findings, e.g. firm zones of the lungs, were interpreted as the result of infection. There were no indications of treatment-related gross necropsy findings. No findings were reported in fasted females at 5000 mg/kg bw. No mortality was observed in fasted male and female rats. One fed male rat of the group at 5000 mg/kg exhibited the following signs starting on day 7 of the study: piloerection, tachypnea (accelerated breathing], stiff/spastic gait, and staggering. The first two signs lasted 1 day. The impaired activity persisted up to day 11 of the study. These clinical signs were not considered to be treatment-related because they were not observed in other fed rats or in fasted rats. No treatment-related effects were observed at necropsy. No mortality was observed in fed male and female rats. The oral LD_{50} in rats (fasted and fed) was > 5000 mg/kg bw (Mihail, 1982).

In a second study, groups of five male and five female Alderley Park (SPF) rats were given triazole alanine (purity not reported) as a single dose at 2000 mg/kg bw by gavage in 20% (w/v) suspension in distilled water. The rats were examined daily for clinical signs and mortality for 14 days. No mortality or clinical signs of toxicity were observed. The oral LD_{50} in rats was > 2000 mg/kg bw (Henderson & Parkinson, 1980).

Mice

Groups of five male and five female NMRI (SPF-Hah) fasted mice were given triazole alanine (purity, 92.8%) as a single dose at 5000 mg/kg bw by gavage in 2.0% Cremophor EL in distilled water. The rats were examined daily for clinical signs and mortality. Treated mice were subjected to gross necropsy at the end of a 14-day observation period. Body weights were recorded at initiation and at the end of the study.

No treatment-related clinical signs, body-weight changes, necropsy findings or mortalities were observed. The oral LD_{50} in mice was > 5000 mg/kg bw (Mihail, 1982).

(b) Administration dermally or by inhalation

No studies were submitted.

(c) Dermal and ocular irritation or sensitization

No studies were submitted.

8.2 Short-term studies of toxicity

Rats

In a 2-week study of oral toxicity, which did not comply with GLP or relevant guidelines, groups of 10 male Bor:WISW (SPF-Cpb) rats were given drinking-water containing triazole alanine (purity, approximately 100%) at a concentration of 0, 3000 or 10 000 ppm. The average daily doses received by the rats were equal to 0, 448 and 1491 mg/kg bw per day, respectively. Cage-side observations were made twice daily, body weights were recorded weekly while food and water consumption were recorded for the entire study period. No opthalmoscopic examinations, urine analysis or haematology and clinical parameter measurements were conducted. All necropsied animals were examined for gross pathology. The weights of the thyroid, thymus, liver, spleen, kidneys, adrenals and brain were determined during gross examination.

Appearance, behaviour, body weight, food consumption, water intake, organ weight, mortality and necropsy findings were unaffected by treatment with drinking-water containing triazole alanine at concentrations up to 10 000 ppm. On the basis of the results of this range-finding study, the study author recommended that concentrations of 1000, 3000 and 10 000 ppm in drinking-water should be tested in short-term study of toxicity.

The NOAEL was > 10 000 ppm, equal to 1491 mg/kg bw per day, the highest concentration tested (Bomhard, 1982).

In a 28-day study of toxicity, which did not comply with GLP, groups of 20 male and 20 female Bor:WISW (SPF-Cpb) rats were given triazole alanine (analytically pure) at a dose of 0, 25, 100 and 400 mg/kg bw per day by gavage suspended in distilled water with added Cremophor EL. After treatment, half the rats from each group were observed for an additional 28 days (recovery period). The rats were inspected daily for signs of toxicity and mortality, with clinical examinations conducted at the end of the 28-day treatment period and at the end of the 4-week recovery period. Body weight and food consumption were measured weekly. At termination, blood was taken for haematological and clinical chemistry analysis. No ophthalmoscopic examination was performed. Urine analysis was performed at the end of the 28-day treatment period and at the end of the 4-week recovery period. At the end of 4 weeks of treatment and on completion of the 4-week recovery period, 10 males and 10 females from each group were sacrificed and subjected to gross pathological examination. Selected tissues from five males and five females in each group were examined histologically and selected organs were weighed.

The test suspension was prepared before each treatment and it was stable for at least 7 h. Results of concentration analysis were not provided.

No biologically or toxicologically significant treatment-related effects were found on food or water consumption, body weight or body-weight gain, and haematology, clinical chemistry or urine-analysis parameters. There were no macroscopic and microscopic changes in tissues and organs. Blood urea and creatinine concentrations (Table 22) were lower in males at 400 mg/kg bw per day and lower urea concentrations were also observed in males at 400 mg/kg bw per day at the end of the recovery period; however, there was no kidney pathology associated with these findings nor were any other clinical parameters affected. Therefore, these effects were not considered to be adverse. Organ-weight analysis revealed lower absolute and relative liver weights for females at 400 mg/kg bw per day (Table 23). This finding was considered spurious in the absence of a corroborating histopathology and clinical chemistry. No induction of total cytochrome P450 was found and the concentration of hepatic triglycerides was not increased.

The NOAEL was > 400 mg/kg per day (the highest dose tested) for males and females exposed to triazole alanine by gavage daily for 28 days (Mihail. & Vogel, 1983).

In a 90-day study of toxicity, groups of 20 male and 20 female Bor:WISW(SPF_Cpb) rats were given diets containing triazole alanine (purity, 97.5%) at a concentration of 0, 1250, 5000 or 20 000 ppm. The average daily doses received by males were 0, 90, 370 and 1510 mg/kg bw per day, respectively and 0, 160, 400 and 1680 mg/kg bw per day for females, respectively. Diets were prepared and stored at room temperature (frequency of preparation not reported). Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for signs of toxicity and mortality, with clinical examinations done after 1 month of treatment and at the end of the study. Body weight and food consumption were measured weekly. Blood was taken for haematological and clinical chemistry analysis after 1 month of treatment and at the end of the study. Urine analysis and ophthalmoscopic examinations were performed before the start of the study and at the end of the study. The selected tissues from all males and females in the control group and the group at 20 000 ppm, and from all animals that died during the study, were examined histologically. In addition, the liver, lung and kidneys and all grossly altered organs or possible target organs from all rats in the groups at 1250 and 5000 ppm were weighed and examined histologically.

Diets were stable for up to 14 days at room temperature. The test article homogeneity results were within the acceptable range (± 12% of nominal). The test substance concentration analysis indicated that the measured test concentrations ranged between 88–116% of the target concentrations.

Males at 20 000 ppm had slightly reduced body weights (approximately 8%) throughout the study and an approximately 11% decrease in total body-weight gain by the end of the study. Minor decreases in leukocyte counts were observed at 20 000 ppm. Clinical chemistry demonstrated changes of no toxicological significance, i.e. statistically significant decreases in triglyceride, bilirubin and urea concentrations in males at 20 000 ppm and in triglyceride concentrations in females at 5000 and 20 000 ppm (Table 24). These changes in haematological and clinical chemistry parameters were considered of no toxicological significance since the changes were small in magnitude, were not seen throughout the study, and were likely to be due to decreases in body-weight gains. No significant treatment-related effects were found for organ weight, haematology, clinical chemistry, urine analysis, or macro- and microscopic tissue examinations.

Table 22. Clinical chemistry parameters (mean values) in male rats given triazole alanine by gavage for 28 days

Parameter	Dose (mg/kg bw per day)							
	0		25		100		400	
	Treatment	Recovery	Treatment	Recovery	Treatment	Recovery	Treatment	Recovery
Urea (mmol/l)	6.65	7.00	6.39	7.25	6.64	5.95	5.35*	5.78*
Creatinine (µmol/l)	61	47	66	48	60	49	49**	52

From TDMG (2008)
* $p \leq 0.05$, ** $p \leq 0.01$.

Table 23. Mean liver weights for female rats given triazole alanine by gavage for 28 days

Liver weight	Dose (mg/kg bw per day)			
	0	25	100	400
Absolute (g)	6.79	6.80	6.52	5.88**
Relative (% of body weight)	3.98	3.88	3.80	3.59**

From TDMG (2008)
** $p < 0.01$

The NOAEL was 5000 ppm, equal to 370 mg/kg bw per day, on the basis of decreased body-weight gains seen at the LOAEL of ≥ 20 000 ppm, equal to 1510 mg/kg bw per day, the highest dose tested (Maruhn & Bomhard, 1984).

Dogs

In a 90-day study of toxicity, four male and four female dogs were given diets containing triazole alanine (purity, 97.5%) at a concentration of 0, 3200, 8000 or 20 000 ppm (equal to 0, 144, 322, and 850 mg/kg bw per day for males and 0, 150, 345, and 902 mg/kg bw per day for females, respectively). Diets were prepared daily. Stability, homogeneity and dietary concentrations were confirmed analytically. The dogs were inspected daily for signs of toxicity and mortality, with clinical observations conducted before the start of the study and in weeks 2, 4, 7 and 13. Body weights were measured weekly and food consumption was measured daily. Before the start of the study and on weeks 2, 4, 7 and 13 blood was taken for haematological and clinical chemistry analysis. Urine analysis was performed before the start of the study and on weeks 2, 4, 7 and 13, while ophthalmoscopic examinations were performed before the start of the study and during weeks 7 and 13. All dogs that died and those that were sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed. Selected tissues were collected for histological examination.

The test-article homogeneity and stability results were within the acceptable range (± 15% of nominal). The analysis of test-substance concentration indicated that the measured test concentration was within 12% of nominal.

No treatment-related deaths or changes in appearance or behaviour occurred during the study. Body temperature, pulse rates, neurological examinations and ophthalmoscopic examinations were not affected by treatment. At the highest dose, female body-weight gain (20% of control) and food consumption (90% of control) were slightly decreased. No haematology or clinical chemistry parameters, gross/histopathology observations, or organ weights showed treatment-related effects. The LOAEL for female dogs was 20 000 ppm, equal to 902 mg/kg bw per day, on the basis of decreased body-weight gains and decrease in food consumption. The corresponding NOAEL for female dogs was 8000 ppm, equal to 345 mg/kg bw per day. The NOAEL for male dogs was > 20 000 ppm, equal to 850 mg/kg bw per day, the highest dose tested (von Keutz & Gröning, 1984).

8.3 Long-term studies of toxicity and carcinogenicity

No studies were submitted.

Table 24. Clinical chemistry parameters (mean values) in male and female rats given diets containing triazole alanine for 90 days

Parameter	Dietary concentration (ppm)															
	0				1250				5000				20 000			
	Males		Females		Males		Females		Males		Females		Males		Females	
	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13
Bilirubin (µmol/l)	2.7	4.4	1.7	3.2	2.5	4.2	1.5	3.2	2.5*	4.1	1.4	2.7	2.0**	3.8**	1.6	3.1
Urea (mmol/l)	8.0	7.6	8.5	7.9	7.6	7.2	8.1	7.6	7.7	6.9	7.3**	7.6	7.7	6.8*	7.5*	7.9
Triglycerides (mmol/l)	1.00	1.22	0.90	1.30	0.98	1.03	0.87	1.24	1.05	1.11	0.61*	0.77**	0.82	0.72**	0.58**	0.85**

From TDMG (2008)
* $p < 0.05$; ** $p < 0.01$

8.4 Genotoxicity

Overall, triazole alanine gave negative results in an adequate battery of assays for genotoxicity in vivo and in vitro. The results of studies of genotoxicity with triazole alanine are summarized in Table 25.

8.5 Reproductive toxicity

(a) Multigeneration studies

In a preliminary study of reproductive toxicity, which did not comply with GLP or relevant guidelines, groups of six male and 12 female Alderley Park rats were given diets containing triazole alanine (purity > 90%) at a concentration of 0, 150, 625, 2500 or 10 000 ppm 6 weeks before mating on a two-

Table 25. Results of studies of genotoxicity with triazole alanine

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Gene mutation[a]	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	20–5 000 µg/0.1 ml ± S9 in DMSO	97.4	Negative	Deparade (1986)
	S. typhimurium TA98, TA100, TA1535 and TA1537	312.5–5 000 µg/plate ± S9 in DMSO	> 96	Negative	Hertner (1993)
	E. coli strain *WP2uvrA*				
	S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538	20–12 500 µg/plate ± S9 in DMSO	Not stated	Negative	Herbold (1983a) [a]
Gene mutation	Chinese hamster V79 cells	500–10 000 µg/0.1 ml ± S9 in water	97.4	Negative	Dollenmeier (1986)
Bacterial DNA repair	*E. coli* pol A+ and pol A1-	62.5–1 000 µg/plate ± S9 in DMSO	NS	Negative	Herbold (1983b) [a]
DNA repair test (rec assay)	*Bacillus subtilis* H17 (rec+) and M45 (rec-)	20–1 000 µg/disk ± S9 in water	> 96	Negative	Watanabe (1993)
Mammalian DNA repair	Rat hepatocytes	80–10 000 µg/ml ± S9 in culture medium	97.4	Negative	Puri (1986)
Mammalian cell transformation	BALB/3T3, clone A31-1-1 mouse fibroblasts	62.5–1 000 µg/ml ± S9 in distilled water	97.4	Negative	Beilstein (1984)
Mammalian cell transformation	Baby hamster kidney (BHK 21 C13) cells	500–8 000 µg/ml −S9 1 000–16 000 µg/ml +S9	NS	Positive	Richold (1981)
In vivo					
Micronucleus formation	Mouse (NMRI)	8 000 mg/kg bw, single oral dose in Cremophore solution	Not stated	Not clastogenic or aneugenic	Herbold (1983c)
	Mouse (CBC F1)	2 500 and 5 000 mg/kg bw, intraperitoneal injection in 0.5% Tween 80	97.4	Not clastogenic or aneugenic	Watkins (1982)
	Chinese hamster	5 000 mg/kg bw, single oral dose in 0.5% carboxymethylcellulose	97.4	Not clastogenic or aneugenic	Strasser (1986)

DMSO, dimethyl sulfoxide; S9, 9000 × *g* supernatant from rodent liver.
[a] Study did not comply with good laboratory practice.
NS = not stated

females-to-one-male basis. The purity of the first batch of triazole alanine was 48% and not > 90% as was originally specified. The dietary concentrations of the second batch using a purer compound were adjusted to the first batch in order to maintain continuous exposure. Male rats were killed after mating and females were allowed to continue on treated diets throughout pregnancy, lactation and weaning of offspring. All parents and selected offspring were subjected to necropsy and selected tissues were examined. Parameters that were recorded or derived during the study included clinical observations, body weight, food consumption and food use during the pre-mating period, body-weight gain in females during pregnancy and in litters from birth to weaning, male and female fertility indices, gestation length, pre-coital interval, live-born index, survival index, litter size and sex distribution.

Diets were stable for up to 10 weeks and homogeneity was satisfactory. The mean achieved dietary concentrations were within 14% of the nominal concentrations except for the second occasion for the dietary concentration of 10 000 ppm (within 21% of the nominal).

No treatment-related clinical signs or mortality were observed. No treatment-related effects on body weight and food consumption were observed. A prolonged pre-coital interval (statistically significant) was noted in rats at 10 000 ppm. No other reproductive parameters were affected by the treatment. At 10 000 ppm, the group-mean litter weight of both male and female pups was significantly reduced on day 1. No other abnormalities in the offspring were noted.

The NOAEL for parental toxicity was 10 000 ppm, equivalent to 1000 mg/kg bw per day, the highest dose tested. The NOAEL for reproductive and offspring toxicity was 2500 ppm, equivalent to 250 mg/kg bw per day, on the basis of increases in pre-coital interval (statistically significant) and the slight reductions in neonatal weights of males and females at 10 000 ppm (Birtley, 1983).

In a two-generation study of reproduction, groups of 15 male and 30 female Alpk:AP(Wistar-derived, SPF) rats were given diets containing triazole alanine (purity, 97.8% w/w) at a concentration of 0, 500, 2000, or 10 000 ppm. The diets were given during premating, mating, gestation and lactation for two successive generations. Two litters were produced in each generation. The F_0 parental rats, aged 4 weeks at the start of premating, received test diets for 12 weeks before they were paired to produce the F_{1a} litters. The F_1 parental animals, aged 5 weeks old when selected at the start of premating, received the test diets for 11 weeks before they were paired to produce the F_{2a} litters. The F_0 and F_1 parents were aged about 16 weeks at mating. After a brief rest period after weaning the first litters, the F_0 and F_1 were re-mated to produce the F_{1b} and F_{2b} generations, respectively. Premating doses of the compound, estimated from graphs, averaged 50, 213 and 1098 mg/kg bw per day, respectively, for F_0 males; 51, 223 and 1109 mg/kg bw per day, respectively, for F_0 females; 47, 192 and 929 mg/kg bw per day, respectively, for F_1 males and 49, 199 and 988 mg/kg bw per day, respectively, for F_1 females. Stability, homogeneity and dietary concentrations were confirmed analytically. Body weights and food consumption were determined on days 1, 8, 15 and 22 of gestation and on days 1, 5, 11, 22 and 29 of lactation. Litter size, number of live and dead pups, individual sexes, weights, and external observations were recorded for pups on the same lactation days. At each dose, rats were randomly selected to continue on treatment as parents for the F_2 generation. They underwent the same study phases as F_0 rats. All F_1 weanlings that were not selected to be parents and all F_2 weanlings were examined as indicated for the culled pups. Adults were necropsied and reproductive tissues were examined histologically.

The test substance was evenly distributed and chemically stable in the diet for approximately 2–3 months. The mean concentrations of test substance were within 10% of the nominal concentrations except for one diet prepared for the group at 10 000 ppm, which was 13% lower than target. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage was acceptable.

A few parental rats died or were killed because of conditions not related to administration of triazole alanine and there were no treatment-related effects on clinical signs, body weight, food

consumption, food use, gross necropsy, microscopic findings, and any measures of reproductive performance. No adverse effects were observed on clinical signs and food consumption, parental body weight, and necropsy findings. No effects were noted on reproductive parameters. There were no treatment-related effects on the following offspring parameters of this study: percentage live-born pups, pup viability, sex ratio, mean litter size, live-birth index, and survival index to day 22. Litter weights and body weights of the F_{1a} group at the highest dose are reduced approximately 10% at all time points, while the F_{1b} offspring were comparable to control values. The F_{2a} litter weights were significantly lower than controls at the intermediate and highest dose; however, the effects were not dose-related. The F_{2b} offspring exhibited decreased mean litter weights in the group at the highest dose. The decreases were statistically and biologically significant at all time points ($p < 0.01$ to $p < 0.05$; decreased 16.3–19.8% in comparison to control values).

The NOAEL for parental systemic toxicity and reproductive toxicity was > 10 000 ppm, equal to 929 mg/kg bw per day, the highest dose tested. The NOAEL for offspring systemic toxicity was 2000 ppm, 192 mg/kg bw per day, on the basis of reduced mean litter weights seen in males and females of both generations at 10 000 ppm, equal to 929 mg/kg bw per day (Milburn et al., 1986).

(b) Developmental toxicity

In a study of developmental toxicity, groups of 24 Alderley Park Alpk/AP (Wistar-derived) female rats were given triazole alanine (purity, 94.8% active ingredient) at a dose of 0, 100, 300 or 1000 mg/kg bw per day by gavage on days 7 to 16 of gestation. Dams were observed for clinical observations, body weight and food consumption. Dams were sacrificed on day 22 of gestation and examined grossly. Each fetus was examined for external malformations including cleft palate. Two thirds of each litter were examined for visceral abnormalities and were subsequently processed for skeletal examination. The remaining fetuses were fixed and decalcified in Bouin fluid for soft tissue examination and serial sectioning of the head.

There were no treatment-related effects on survival, clinical signs, body weight or body-weight gain, food consumption or caesarian parameters. The number of fetuses (litters) examined externally and viscerally was 279 (24), 301 (24), 264 (24) and 293 (24) and the number examined skeletally was 185 (24), 201 (24), 178 (24) and 195 (24) in the control group and groups at the lowest, intermediate and highest dose, respectively (Table 26 and Table 27). A statistically significant increase in the fetal incidence of several skeletal anomalies was found in the group at the highest dose. The fetal (litter) incidence rates in the control group and groups at the lowest, intermediate, and highest dose were 95 (23), 95 (23), 92 (23) and 122 (23), respectively, for total minor skeletal findings, 1 (1), 3 (2), 2 (2) and 12 (7), respectively, for partially ossified transverse processes of the seventh cervical vertebra (bilateral), 0 (0), 0 (0), 1 (1) and 6 (2), respectively, for unossified fifth sternebra, and 1 (1), 4 (2), 4 (4) and 7 (6), respectively, for partially ossified 13th thoracic centrum. In addition, the fetal incidence of unossified odontoid process was increased in groups at the intermediate and highest dose, 12 (9), 6 (6), 24 (13) and 29 (15), respectively. In the absence of historical control data, these skeletal anomalies were considered to be adverse.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day on the basis of the absence of adverse findings at this dose, the highest tested. The NOAEL for developmental toxicity was 100 mg/kg bw per day on the basis of an increased incidence of non-ossification of the odontoid process (delayed ossification) at 300 mg/kg bw per day. Triazole alanine did not induce teratogenicity at up to the highest dose of 1000 mg/kg bw per day (Clapp et al., 1983).

8.6 Special studies

No studies were submitted.

Studies on metabolites were not submitted. On the basis of results of the metabolism-identification study, no studies were necessary since the major compound identified was triazole alanine itself.

9. Observations in humans

Observations in humans were not submitted.

Table 26. Incidence of external and visceral effects in a study of developmental toxicity in rats given triazole alanine by gavage

Observations	Dose (mg/kg bw per day)			
	0	100	300	1000
Fetuses (litters) examined	279 (24)	301 (24)	264 (24)	293 (24)
Fetuses (litters) affected—minor defects	13 (7)	5 (5)	12 (8)	7 (5)
Fetuses (litters) affected—major defects	0	0	0	1 (1)
Unilateral increased renal pelvic dilatation-slight (minora)	10 (5)[b]	4 [c] (4)	8 (6)	4 (3)
Severely malformed fetus (major)	0	0	0	1 (1)
Bilateral increased renal pelvic dilatation-slight (minor)	2 (2)	0	2 (2)	1 (1)
Left gonad vestigial (minor)	0	0	0	1 (1)

From Clapp et al. (1983)

[a] Moderate dilatation was seen in one pup at the intermediate dose.

[b] Fetal (litter) incidence

[c] The summary table indicated that five fetuses had this finding; however, this reviewer could only find four.

Table 27. Skeletal effects in a study of developmental toxicity in rats given triazole alanine by gavage

Observations	Dose (mg/kg bw per day)			
	0	100	300	1000
Fetuses (litters) examined	185 (24)	201 (24)	178 (24)	195 (24)
Fetuses (litters) affected—minor defects	95 (23)	95 (23)	92 (23)	122* (23)
Fetuses (litters) affected—major defects	0	0	0	1 (1)
Fetuses (litters) affected—variants	170 (23)	197** (24)	169 (24)	186 (24)
Transverse processes of seventh cervical vertebra-bilateral- partially ossified (minor)	1 (1)[a]	3 (2)	2 (2)	12** (7)
Fifth sternebra not ossified (minor)	0	0	1 (1)	6[b] (2)
Odontoid process not ossified (minor)	12 (9)	6[c] (6)	24* (13)	29** (15)
Thirteenth thoracic centrum partially ossified (minor)	1 (1)	4 (2)	4 (4)	7* (6)

From Clapp et al. (1983)

[a] Fetal (litter) incidence.

[b] The summary table indicates that seven fetuses had this finding and based on this incidence it was statistically higher than the control group; however, this reviewer could only find six fetuses with this finding which is also likely to be significantly higher. It is possible this finding was observed in the fetus that was severely malformed; however, from the description of that fetus, it cannot be confirmed.

[c] This reviewer could only find six fetuses with odontoid process not ossified. The report indicated that the incidence was 10.

*$p < 0.05$; ** $p < 0.01$.

Comments

1,2,4-TRIAZOLE

Biochemical aspects

In rats treated orally, radiolabelled 1,2,4-triazole was rapidly and completely absorbed and excreted mostly unchanged and mainly in the urine (80–94%) in the first 24 h, irrespective of dose or route of administration. Approximately 0.1% of the administered dose was recovered within 30 h in expired air after oral and intravenous administration. Approximately 3–5% of the administered dose was recovered in faeces in 48 h. Approximately 2% of the administered dose was recovered in the gastrointestinal tract at 48 h. In bile duct-cannulated rats, approximately 12% of the dose was recovered in the bile at 24 h after intravenous or intraduodenal application.

Toxicological data

1,2,4-Triazole is of moderate toxicity when administered orally. The LD_{50} in rats treated orally was 1648 mg/kg bw. The LD_{50} in rats treated dermally was 3129 mg/kg bw. 1,2,4-Triazole appears to be more toxic dermally in rabbits than in rats. The dermal LD_{50} in rabbits was > 200 and < 2000 mg/kg bw. It is slightly irritating to the skin and severely irritating to the eyes of rabbits. It is not a skin sensitizer as determined by Magnusson & Kligman (maximization) test in guinea-pigs. The following clinical signs were observed after oral dosing: sedation, breathing difficulties, reduction in general well-being, hunched posture (at higher doses). These signs appeared within 1 h of administration and were observed for a maximum of 13 days after administration. Similar clinical signs were observed in rats treated dermally.

In short-term studies in mice and rats, neurotoxicity was seen in number of studies. In a 28-day study of toxicity in mice, the only treatment-related effects were slight testicular degeneration accompanied by apoptotic bodies at 2000 ppm, equal to 356 mg/kg bw per day (the LOAEL). No effects were observed in females at doses up to and including 2000 ppm, equal to 479 mg/kg bw per day. The NOAEL in mice was 500 ppm, equal to 90 mg/kg bw per day.

In a 90-day study of toxicity in mice, decreased body weight, tremors (observed from day 30) and loss of cerebellar Purkinje cells were observed in males and females at 6000 ppm, equal to 988 mg/kg bw per day. At 6000 ppm (the highest dose), 9 out of 11 males showing tremors also had Purkinje cell loss, while in females at this highest dose one out of three mice with tremors had Purkinje cell loss. Decreased testicular weights and histopathological findings in testes similar to the 28-day study were observed in males at 3000 and 6000 ppm. The NOAEL was 1000 ppm, equal to 161 mg/kg bw per day, on the basis of tremors, decreased brain weights, decreased testicular weights and histopathological changes in the testes seen in males at the LOAEL of 3000 ppm, equal to 487 mg/kg bw per day.

In a 90-day dietary study of toxicity in rats, retarded body-weight development, transient effects on the central nervous system, lower erythrocyte parameters (microcytic hypochromic erythrocytes, in males only) and hepatocellular fat accumulation (males only) were observed at 2500 ppm, equivalent to 212.3 mg/kg bw per day. The NOAEL was 500 ppm, equivalent to 37.9 mg/kg bw per day. In a combined short-term study of toxicity and neurotoxicity in rats, FOB effects were observed at 3000 ppm and 1000/4000 ppm (equal to 183 and 210 mg/kg per day, respectively) and with increased incidence and severity at week 8. Males were more severely affected than females. Other effects observed were ungroomed appearance, red nasal and lachrymal stain, yellow urine stain, muscle fasciculations, tremors, gait incoordination, decreased activity in the open field, decreased rearing, uncoordinated righting reflex and increased foot splay. A decrease in motor and locomotor activity was also observed in males at 3000 ppm during week 4 only. Decreases in absolute brain weights and degenerative lesions were seen in the cerebellum, the lumbar dorsal root ganglion and

other peripheral nerves at 3000 ppm and at 1000/4000 ppm. The brain lesions were limited to the anterior, dorsal cerebellum and were coded overall as an increased incidence of cellular degeneration and necrosis. Findings were characterized by extensive loss of Purkinje cells, variable white-matter degeneration and gliosis. Subtle atrophy of the molecular layer, primarily at the cerebellar surface, or loss of granule cells was occasionally present. The NOAEL was 500 ppm, equal to 33 mg/kg bw per day, on the basis of decreased body weight and body-weight gain, tremor and incoordination, decreased absolute brain weight, and increased incidence of neuropathology findings in the peripheral and central nervous system at the LOAEL of 3000 ppm, equal to 183 mg/kg bw per day.

1,2,4-Triazole gave negative results in a battery of assays for genotoxicity, including the Ames test in vitro, an assay for forward mutation, and a test for chromosomal aberration.

The Meeting concluded that 1,2,4-triazole is unlikely to be genotoxic.

No studies of carcinogenicity were submitted. However, the Meeting considered that 1,2,4-triazole is unlikely to be carcinogenic at anticipated levels of exposure since it does not accumulate in the body, it is non-mutagenic, and because of the absence of pre-neoplastic changes with 1,2,4-triazole at high doses.

In a two-generation study of reproductive toxicity in rats, decreased body weights were observed in F_1 males at 250 ppm, equal to 16 mg/kg bw per day, the lowest dose tested. These changes in body weight were minor and were seen only in males and in only one generation and were not seen in short-term studies in rats given similar doses. At 3000 ppm, parental animals (F_0) had statistically significantly reduced terminal body weights, and decreased absolute brain weights associated with mild to moderate degeneration/necrosis in the cerebellum. No F_1 offspring at the highest dose survived the lactation period. No offspring toxicity was observed at doses up to 500 ppm, equal to 30.9 mg/kg bw per day. The NOAEL for reproductive toxicity with 1,2,4-triazole was 250 ppm, equal to 16 mg/kg bw per day, on the basis of an increase in abnormal sperm in F_0 and F_1 males seen at the LOAEL of 500 ppm.

In two studies of developmental toxicity in rats, there was maternal toxicity (retarded weight gain) at 100 mg/kg bw per day or higher, developmental toxicity (decreased body weights, lower fetal and placental weights, and a higher incidence of minor skeletal deviations) at 100 mg/kg bw per day or higher, and an increased incidence of malformations (hydronephrosis, cleft palate, long-bone dysplasia, diaphragmatic hernia) at 200 mg/kg bw per day. The NOAEL for maternal toxicity and for developmental toxicity in rats was 30 mg/kg bw per day. In a study in rabbits, however, lower body-weight gain and clinical signs of systemic toxicity such as excess salivation, hyperpnoea and ptosis were evident at 45 mg/kg bw per day. Five out of 25 dams at this dose were sacrificed in a moribund condition. Developmental effects included lower body weights of fetuses at 45 mg/kg bw per day, and there were a few alterations in the urogenital system, which occurred in several fetuses. The NOAEL for maternal toxicity and for developmental toxicity was 30 mg/kg bw per day in rabbits.

The Meeting concluded that 1,2,4-triazole is teratogenic in rats and rabbits at maternally toxic doses.

No study of acute neurotoxicity was submitted. Clinical signs of neurotoxicity were observed in studies of acute toxicity in which very high doses were given dermally or orally. Neurotoxic effects observed in a short-term study of combined toxicity/neurotoxicity are described above.

The Meeting concluded that 1,2,4-triazole is neurotoxic.

The Meeting concluded that the existing database on 1,2,4-triazole was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.2 mg/kg bw based on a NOAEL of 250 ppm, equal to 16 mg/kg per day, on the basis of testicular effects (sperm abnormalities, sperm counts) seen at

500 ppm, equal to 30.9 mg/kg bw per day, and using a safety factor of 100. At 250 ppm, reduced body weights and body-weight gains were observed in F_1 males; however, the Meeting noted that the reductions in body weight observed at 250 ppm were marginal (< 6%) and were seen only in one sex and in only one generation and were not seen in short-term studies with similar doses. The Meeting therefore concluded that it was not necessary to use an additional safety factor. This ADI is protective for neurotoxic effects seen at 3000 ppm, equal to 183 mg/kg bw per day, in a short-term study of toxicity/neurotoxicity in rats in which the NOAEL was 500 ppm, equal to 33 mg/kg bw per day. The Meeting considered that it was not necessary to add an additional safety factor to allow for the lack of studies of carcinogenicity because 1,2,4-triazole is unlikely to be carcinogenic at anticipated levels of exposure since it does not bioaccumulate in the body, it is non-mutagenic, and because of the absence of pre-neoplastic changes at high doses.

The Meeting established an ARfD of 0.3 mg/kg bw based on a NOAEL of 30 mg/kg bw per day, identified on the basis of alterations of the urogenital system that occurred in several fetuses at the LOAEL of 45 mg/kg bw per day and clinical signs of neurotoxicity in the dams in a study of developmental toxicity in rabbits, and using a safety factor of 100.

Levels relevant to risk assessment for 1,2,4-triazole

Species	Study	Effect	NOAEL	LOAEL
Mouse	Ninety-day study of toxicity[a]	Toxicity	1000 ppm, equal to 161 mg/kg bw per day	3000 ppm, equal to 487 mg/kg bw per day
Rat	Ninety-day study of toxicity[a]	Toxicity	500 ppm, equal to 33 mg/kg bw per day	3000 ppm, equal to 183 mg/kg bw per day
	Multigeneration study of reproductive toxicity[a]	Parental toxicity	250 ppm, equal to 16.0 mg/kg bw per day[d]	500 ppm, equal to 31 mg/kg bw per day[c]
		Offspring toxicity	500 ppm, equal to 31 mg/kg bw per day[c]	—
	Developmental toxicity[b]	Maternal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	30 mg/kg bw per day	45 mg/kg bw per day[c]
		Embryo and fetal toxicity	30 mg/kg bw per day	45 mg/kg bw per day[c]

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.
[d] Marginal effects on body weight, only seen in F_1 males.

Estimate of acceptable daily intake for humans

0–0.2 mg/kg bw per day

Estimate of acute reference dose

0.3 mg/kg bw

Information that would be useful for continued evaluation of the compound

Results from epidemiological and other such observational studies of human exposures

Critical end-points for setting guidance values for exposure to 1,2,4-triazole

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid and nearly complete absorption
Distribution	Widely distributed in tissues
Potential for accumulation	Low, no evidence of significant accumulation
Rate and extent of excretion	Approximately 80–94% of the administered dose excreted in urine in first 24 h
Metabolism in animals	No significant metabolism
Toxicologically significant compounds (animals, plants and environment)	1,2,4-Triazole

Acute toxicity

Rat, LD_{50}, oral	1650 mg/kg bw
Rat, LD_{50}, dermal	3129 mg/kg bw
Rat, LC_{50}, inhalation	No adequate data
Rabbit, dermal irritation	Slight irritation
Rabbit, ocular irritation	Severe irritation
Guinea-pig, dermal sensitization	Not a sensitizer (Magnusson & Kligman test)

Short-term studies of toxicity

Target/critical effect	Nervous system, brain
Lowest relevant oral NOAEL	500 ppm, equal to 33 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEL	No data

Genotoxicity

	Unlikely to be genotoxic

Long-term studies of toxicity and carcinogenicity

Target/critical effect	No data
Lowest relevant NOAEL	No data
Carcinogenicity	Unlikely to be carcinogenic

Reproductive toxicity

Reproduction target/critical effect	Sperm abnormalities, decrease in body weight
Lowest relevant reproductive NOAEL	250 ppm, equal to 16 mg/kg bw per day
Developmental target/critical effect	Urogenital alterations in rabbits
Lowest relevant developmental NOAEL	30 mg/kg bw per day (rats and rabbits)

Neurotoxicity/delayed neurotoxicity

Neurotoxicity	Evidence of clinical signs of neurotoxicity and cerebellar lesions

Mechanistic data

No studies were submitted

Medical data

No data

Summary

	Value	Study	Safety factor
ADI	0–0.2 mg/kg bw per day	Rat, two-generation studies of reproductive toxicity	100
ARfD	0.3 mg/kg bw	Rabbit, study of developmental toxicity	100

TRIAZOLE ALANINE AND TRIAZOLE ACETIC ACID

Biochemical aspects

In rats given a single dose of radiolabelled triazole alanine (up to 994 mg/kg bw) by gavage, almost all the administered dose was absorbed on the basis of urinary excretion (69–98%). Approximately 3–18% of the administered dose was recovered in the faeces after 7 days. Less than 0.5% of the administered dose was recovered in the expired air. No significant bioaccumulation of triazole alanine was observed. Approximately 8–30% of the excreted dose in the urine and < 1% of the dose in faeces was identified as *N*-acetyl-D,L-triazole alanine, the remainder was parent compound.

In rats given a single dose of radiolabelled triazole acetic acid by gavage, almost all the administered dose (96–112%) was absorbed on the basis of urinary excretion. Triazole acetic acid was rapidly absorbed and excreted mainly via the urine (87–104% after 7 days). Approximately 1.2–7.4% of the administered dose was recovered in the faeces after 7 days. Total radiolabel in tissues after 7 days ranged from 0.8% to 3.1% of the administered dose. Only the parent compound was found in the urine.

Toxicological data

Triazole alanine is of low acute toxicity when administered orally. The oral LD_{50} in mice and rats was > 5000 mg/kg bw. No treatment-related clinical signs or mortalities were observed in these studies.

Triazole acetic acid is of low acute toxicity when administered orally. The oral LD_{50} in rats was > 5000 mg/kg bw. A slight to moderate increase in the incidence of dyspnoea, exophthalmos, ruffled fur, and hunched posture were observed after dosing and subsided within 10 days.

For triazole alanine, no target organ or any treatment-related toxicity was observed in short-term studies in rats and dogs, except for reduced body-weight gains observed in 90-day studies of toxicity in rats and dogs (females only). No long-term studies were submitted.

For triazole acetic acid, no target organ or any treatment-related toxicity was observed in a short-term study in rats. No long-term studies were submitted.

No treatment-related toxicity was observed in a 14-day study in rats given drinking-water containing triazole alanine at concentrations up to 10 000 ppm, equal to 1491 mg/kg bw per day. Haematological and clinical chemistry parameters were not measured in this study. No treatment-related effects were seen in the 28-day study of oral toxicity in which rats were given triazole alanine at doses of up to 400 mg/kg bw per day by oral gavage. In this study, haematological, clinical chemistry and histopathological analyses were incomplete.

In a 90-day dietary study of toxicity in rats fed triazole alanine, decreased body-weight gains was observed at the highest dose of 20 000 ppm, equal to 1510 mg/kg bw per day. Small decreases in concentrations of leukocytes, triglycerides and bilirubin were observed, but were considered to be of no toxicological significance since the changes were small and may have been secondary to the decreased body weights. The NOAEL was 5000 ppm, equal to 370 mg/kg bw per day.

In a 90-day dietary study of toxicity in dogs fed triazole alanine, decreased body-weight gain and food consumption was observed in females at the highest dose of 20 000 ppm, equal to 902 mg/kg bw per day. The NOAEL was 8000 ppm, equal to 322 mg/kg bw per day.

No treatment-related toxicity was observed in a 14-day study in rats given diets containing triazole acetic acid at doses of up to 8000 ppm, equal to 703.5 mg/kg bw per day.

Triazole alanine gave negative results in a adequate battery of tests for genotoxicity in vivo and in vitro.

Triazole acetic acid gave negative results in an Ames test in vitro, and in assays for mutation or cytogenotoxicity in mammalian cells.

The Meeting concluded that triazole alanine and triazole acetic acid are unlikely to be genotoxic.

No studies of carcinogenicity were available; however, triazole alanine and triazole acetic acid are unlikely to be carcinogenic at anticipated levels of exposure since they do not bioaccumulate in the body, are non-mutagenic, are not chemically reactive, and no specific target-organ toxicity was identified in the available toxicological studies with doses of up to 1510 mg/kg bw per day.

In a non-guideline, one-generation study of reproductive toxicity in rats given triazole alanine, no systemic toxicity was seen in parental animals at doses of up to 10 000 ppm, equivalent to 1000 mg/kg bw per day. In this study, a statistically significant increase in pre-coital interval and slight reductions in neonatal weights of males and females were observed at 10 000 ppm. The NOAEL for reproductive and developmental toxicity was 2500 ppm, equal to 250 mg/kg bw per day. In a two-generation study of reproductive toxicity in rats, no systemic toxicity was observed in the parental animals at doses of up to and including 10 000 ppm. No reproductive toxicity was observed at doses of up to and including 10 000 ppm, equal to 929 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 2000 ppm, equal to 192 mg/kg bw per day, on the basis of reduced mean litter weights seen at the LOAEL of 10 000 ppm, equal to 929 mg/kg bw per day. In a study of developmental toxicity in rats given triazole alanine, no systemic toxicity was observed with triazole alanine at doses of up to and including 1000 mg/kg bw per day given by oral gavage. Increased incidences of skeletal findings were seen in the offspring at the intermediate and highest doses. These skeletal findings included unossified odontoid processes at 300 and 1000 mg/kg bw per day, with partially ossified transverse processes of the seventh cervical vertebra (bilateral), unossified fifth sternebra, and partially ossified 13th thoracic centrum observed only at 1000 mg/kg bw per day. The NOAEL for developmental toxicity was 100 mg/kg bw per day.

The Meeting concluded that triazole alanine was not teratogenic. Triazole acetic acid is unlikely to be teratogenic on the basis of its structural and toxicological similarity with triazole alanine.

No studies of neurotoxicity with triazole alanine were submitted. However, there was no evidence that exposure to triazole alanine results in neurotoxicity in the short-term studies in rats and dogs, the study of developmental toxicity in rats, or studies of reproductive toxicity in rats.

No studies of neurotoxicity with triazole acetic acid were submitted. In a study of acute lethality, a slight to moderate increase in the incidence of dyspnoea, exophthalmos, ruffled fur, and curved body position were observed after dosing, and subsided within 10 days. These clinical signs were considered to be non-specific and attributable to bolus dosing with a very high dose (5000 mg/kg bw) by gavage rather than specific neurotoxicity.

The Meeting concluded that triazole alanine and triazole acetic acid are unlikely to be neurotoxic on the basis of the available data.

The Meeting concluded that the existing database on triazole alanine was adequate to characterize the potential hazards to fetuses, infants and children. This conclusion was also applicable to triazole acetic acid for the reasons described above.

Toxicological evaluation

The Meeting established a group ADI for triazole alanine and triazole acetic acid (alone or in combination) of 0–1.0 mg/kg bw based on a NOAEL of 100 mg/kg bw per day for developmental toxicity in a study of developmental toxicity in rats given triazole alanine, on the basis of delayed ossification seen in rats at the LOAEL of 300 mg/kg bw per day, and using a safety factor of 100. The Meeting concluded that it was not necessary to use an additional safety factor for the lack of studies of carcinogenicity because the compounds are unlikely to be carcinogenic at anticipated levels of exposure, do not bioaccumulate in the body, are non-mutagenic, are not chemically reactive, and no specific target-organ toxicity was identified in the available toxicological studies with doses of up to 1510 mg/kg bw per day.

The Meeting concluded that it was unnecessary to establish an ARfD for triazole alanine and triazole acetic acid because no toxicity could be attributed to a single exposure in the available database, including a study of developmental toxicity in rats.

Levels relevant to risk assessment for triazole alanine and triazole acetic acid (based on data for triazole alanine

Species	Study	Effect	NOAEL	LOAEL
Rat	Multigeneration study of reproductive toxicity[a]	Parental toxicity	10 000 ppm, equal to 929 mg/kg bw per day[c]	—
		Offspring toxicity	2 000 ppm equal to 192 mg/kg bw per day	10 000 ppm, equal to 929 mg/kg bw per day[c]
	Developmental toxicity[b]	Maternal toxicity	1 000 mg/kg bw per day[c]	—
		Embryo and fetal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
Dog	Ninety-day study of toxicity[b]	Toxicity	8 000 ppm, equal to 345 mg/kg bw per day	20 000 ppm, equal to 850 mg/kg bw per day[c]

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

Estimate of acceptable daily intake for humans

Group ADI for triazole alanine and triazole acetic acid: 0–1 mg/kg bw per day

Estimate of acute reference dose

Unnecessary

Information that would be useful for continued evaluation of the compound

Results from epidemiological and other such observational studies of human exposure.

Critical end-points for setting guidance values for exposure to triazole alanine and triazole acetic acid

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid and nearly complete absorption
Distribution	Widely distributed in tissues
Potential for accumulation	Low, no evidence of significant accumulation
Rate and extent of excretion	Approximately 96–99% of the administered dose excreted in urine in first 24 h
Metabolism in animals	Limited, about 8–19% excreted as N-acetyl triazole alanine in the urine. No metabolism of triazole acetic acid.
Toxicologically significant compounds (animals, plants and environment)	Triazole alanine; triazole acetic acid

Acute toxicity

Rat, LD_{50}, oral	> 5000 mg/kg bw for triazole alanine and triazole acetic acid
Rat, LD_{50}, dermal	No data
Rat, LC_{50}, inhalation	No data
Rabbit, dermal irritation	No data
Rabbit, ocular irritation	No data
Dermal sensitization	No data

Short-term studies of toxicity

Target/critical effect	Decreased body-weight gain
Lowest relevant oral NOAEL	5000 ppm, equal to 370 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEL	No data

Genotoxicity

	Unlikely to be genotoxic (triazole alanine and triazole acetic acid)

Long-term studies of toxicity and carcinogenicity

Target/critical effect	No data
Lowest relevant NOAEL	No data
Carcinogenicity	Unlikely to be carcinogenic (triazole alanine and triazole acetic acid)

Reproductive toxicity

Reproduction target/critical effect	No toxicologically relevant effects
Lowest relevant reproductive NOAEL	10 000 ppm, equal to 929 mg/kg bw per day (rats; highest dose tested)
Developmental target/critical effect	Delayed ossifications
Lowest relevant developmental NOAEL	100 mg/kg bw per day (rats)

Neurotoxicity/delayed neurotoxicity

Acute neurotoxicity	No indication of neurotoxicity from other studies

Mechanistic data

No data

Medical data

No data

Summary

	Value	Study	Safety factor
ADI	0–1 mg/kg bw per day	Rat, study of developmental toxicity	100
ARfD	Unnecessary	—	—

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ANNEX 1

Reports and other documents resulting from previous Joint Meetings Of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and 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.

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This volume contains toxicological monographs that were prepared by the 2008 Joint FAO/WHO Meeting on Pesticide Residues (JMPR), which met in Rome on 18–27 September, 2008.

The monographs in this volume summarize the safety data on 10 pesticides that could leave residues in food commodities. These pesticides are azoxystrobin, buprofezin, carbofura, chlorantraniliprole, hexythiazoz, mandipropamid, prothioconazole, spinetoram, spirotetramat and triazole fungicide metabolites. 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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