Pesticide residues in food — 2019

Extra Joint FAO/WHO Meeting on Pesticide Residues

EVALUATIONS 2019

Part II — Toxicological

Food and Agriculture Organization of the United Nations

World Health Organization

Pesticide residues in food – 2019

Toxicological evaluations

Sponsored jointly by FAO and WHO

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

Gatineau, Canada, 7–17 May 2019

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TABLE OF CONTENTS

2019 Extra Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues

Gatineau, Canada, 7–17 May 2019

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Abbreviations used

4-HPPD	4-hydroxyphenylpyruvate dioxygenase
5-OH-dicamba	2,5-dichloro-3-hydroxy-6-methoxybenzoic acid
ADI	acceptable daily intake
AFC	antibody-forming cell
AMBA	2-amino-4-methylsulfonylbenzoic acid
AR	applied radioactivity
ARfD	acute reference dose
AUC	area under the concentration–time curve
bw	body weight
CHO	Chinese hamster ovary
C_{max}	maximum concentration
CMC	carboxymethylcellulose
CYP	cytochrome P450
DCGA	3,6-dichlorogentisic acid
DCSA	3,6-dichlorosalicylic acid
DMBA	7,12-dimethylbenz[*a*]anthracene
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EC_{50}	median effective dose
EMS	ethyl methanesulfonate
equiv	equivalent
EU	European Union
Exp.	experiment
FAO	Food and Agriculture Organization of the United Nations
FOB	functional observational battery
GLP	good laboratory practice
HPLC	high-performance liquid chromatography
Hprt	hypoxanthine–guanine phosphoribosyltransferase
IC_{50}	median inhibitory concentration
IgM	immunoglobulin M
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC	liquid chromatography
LD_{50}	median lethal dose
LMA	locomotor activity
LOAEL	lowest-observed-adverse-effect level
MNBA	2-nitro-4-methylsulfonylbenzoic acid
MS	mass spectrometry
MS/MS	tandem mass spectrometry
m/*z*	mass-to-charge ratio
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
nd	not detected
NE	not examined
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NRU	neutral red uptake
NS	not specified
NZW	New Zealand white
OECD	Organisation for Economic Co-operation and Development
PCE	polychromatic erythrocytes
PEG	polyethylene glycol
PIF	photoirritation factor

PND	postnatal day
ppm	parts per million
QSAR	quantitative structure–activity relationship
ROI	region of interest
RRT	relative retention time
S9	9000 × *g* supernatant fraction from rat liver homogenate
SD	standard deviation; Sprague Dawley
SN	scheduled necropsy
T_3	triiodothyronine
T_4	thyroxine
TK	thymidine kinase
T_{max}	time to reach maximum concentration
TSH	thyroid stimulating hormone
TTC	threshold of toxicological concern
U	uniformly labelled
UD	unscheduled death
USA	United States of America
UVA	ultraviolet A
WHO	World Health Organization
WI	Wistar
w/v	weight per volume
w/w	weight per weight

Introduction

The toxicological monograph addenda contained in this volume were prepared by the WHO Core Assessment Group on Pesticide Residues, which met with the FAO Panel of Experts on Pesticide Residues in Food and the Environment in an Extra Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in Gatineau, Canada, on 7–17 May 2019.

Follow-up evaluations were conducted on all eight compounds considered by the WHO Core Assessment Group (acetochlor, boscalid, chlorothalonil, cyprodinil, dicamba, mesotrione, metaflumizone and thiabendazole). Reports and other documents resulting from previous Joint FAO/WHO Meetings on Pesticide Residues are listed in Annex 1.

The report of the Extra Joint Meeting has been published by FAO under the title *Pesticide residues in food – 2019. Report of the Extra Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues*. That report contains comments on the compounds considered, reconsideration of previously established acceptable daily intakes and acute reference doses in light of data newly submitted to the WHO Core Assessment Group, and maximum residue levels established by the FAO Panel of Experts. Monographs on residues prepared by the FAO Panel of Experts are published as a companion volume, under the title *Pesticide residues in food – 2019 evaluations. Part I. Residues*.

The toxicological monograph addenda contained in this volume are based on working papers that were prepared by WHO experts before the 2019 Extra Joint Meeting. A special acknowledgement is made to those experts and to the experts of the Extra Joint Meeting who reviewed early drafts of these working papers.

These monograph addenda were prepared based on evaluations of the original studies and the dossiers provided by the sponsors of the compounds, of the relevant published scientific literature and of the data submitted by Codex members. When found consistent with the data of the original studies, the monograph addenda may contain parts of the text and tables of the dossiers submitted by the sponsors, but not the conclusions of the sponsors. These monograph addenda and their conclusions are based on independent reviews of the available data and do not constitute an endorsement of the position of the sponsors.

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

TOXICOLOGICAL
MONOGRAPH
ADDENDA

ACETOCHLOR (addendum)

First draft prepared by
Midori Yoshida[1] and P.V. Shah[2]

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Explanation

Acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide) was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2015, when an acceptable daily intake (ADI) of 0–0.01 mg/kg body weight (bw) and an acute reference dose (ARfD) of 1 mg/kg bw were established (Annex 1, reference *136*).

Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, acetochlor was placed on the agenda of the present Meeting, which assessed additional toxicological information available since the last review.

In order to predict the genotoxicity of metabolite A (acetochlor *tert*-sulfinyllactic acid) and metabolite B (acetochlor 1-hydroxyethyl *sec*-oxanilic acid), which are soybean metabolites, the sponsor provided in silico data on general toxicity and genotoxicity for acetochlor, metabolites A and B and related metabolites considered by the 2015 Meeting. The present Meeting applied this information to the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015) for metabolites A and B.

Evaluation for acceptable intake

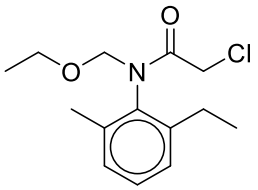
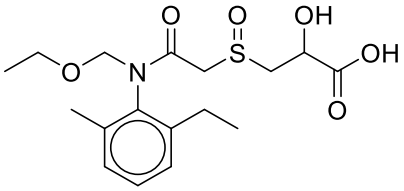
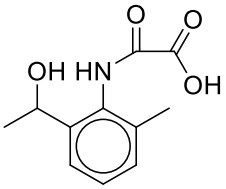
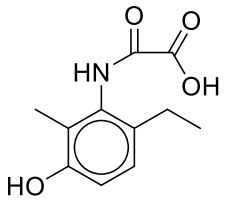
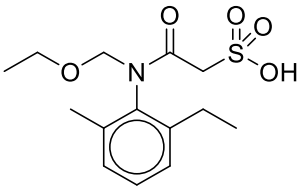
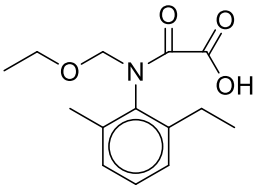
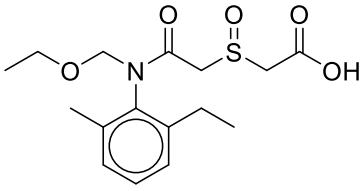
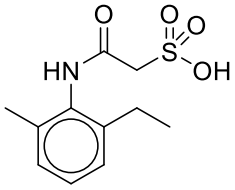
As the current evaluation is based on quantitative structure–activity relationships (QSARs) and read-across, the chemical structures of acetochlor, metabolites A and B and the metabolites evaluated by the previous Meeting (Annex 1, reference *136*) are shown in Table 1.

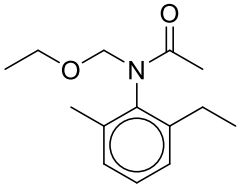
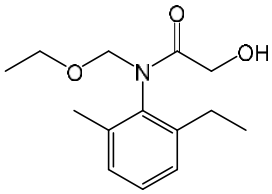
1. Toxicological studies on metabolites

1.1 General toxicity of metabolites A and B

The information provided to the Meeting on the general toxicity of metabolites A and B was purely qualitative in nature and did not permit any robust quantitative conclusions on the toxicity of these metabolites relative to that of acetochlor.

Table 1. Chemical structures of acetochlor, metabolites A and B and the metabolites evaluated by the 2015 Meeting

Name (abbreviation)	Structure
Acetochlor	
Acetochlor *tert*-sulfinyllactic acid (metabolite A)	
Acetochlor 1-hydroxyethyl *sec*-oxanilic acid (metabolite B)	
Acetochlor *N*-(6-ethyl-3-hydroxy-2-methylphenyl)oxamic acid	
Acetochlor *tert*-ethanesulfonic acid (*t*-ESA) (EU reference no. 7)	
Acetochlor *tert*-oxanilic acid (*t*-OXA) (EU reference no. 2)	
Acetochlor *tert*-sulfinylacetic acid (*t*-SAA) (EU reference no. 3)	
Acetochlor *sec*-ethanesulfonic acid (*s*-ESA) (EU reference no. 13)	

Name (abbreviation)	Structure
tert-Norchloroacetochlor (*t*-NCA) (EU reference no. 6)	
tert-Hydroxyacetochlor (EU reference no. 17)	

EU: European Union
Source: Saltmiras (2018)

1.2 Genotoxicity of metabolites A and B

The 2015 Meeting concluded that acetoclor and the metabolites that were evaluated at that meeting were unlikely to pose a concern for genotoxicity in vivo (Annex 1, reference *136*).

In silico predictions of the genotoxicity of metabolites A and B (Pellizzaro & Beevers, 2018) were submitted to the present Meeting. The predictions were generated using Derek Nexus, Leadscope and the Organisation for Economic Co-operation and Development (OECD) QSAR Toolbox and were prepared in accordance with guidance on the establishment of the residue definition for dietary risk assessment (EFSA, 2016).

Metabolite A was concluded to be non-genotoxic on the basis of modelling by both Derek Nexus and Leadscope, the lack of genotoxicity alerts of potential concern identified by the OECD QSAR Toolbox and a literature search of substances containing the hydroxycarboxylic acid functional group, which found only non-genotoxic substances.

Metabolite B was concluded to be non-genotoxic on the basis of modelling by Derek Nexus and Leadscope and the lack of genotoxicity alerts of potential concern identified by the OECD QSAR Toolbox.

Comments

Toxicological data on metabolites

In silico predictions of the general toxicity and genotoxicity of metabolites A and B were submitted to the present Meeting. The predictions were prepared using Derek Nexus, Leadscope and the OECD QSAR Toolbox.

Based on their structural similarity to the metabolites of acetochlor evaluated by the 2015 JMPR, metabolites A and B were predicted to be less toxic than acetochlor on the basis of likely lower systemic absorption following oral exposure, rapid excretion, minimal metabolism and lack of tissue distribution or localization.

Metabolite A was predicted to be non-genotoxic on the basis of modelling using both Derek Nexus and Leadscope, a lack of genotoxicity alerts of potential concern identified by the OECD QSAR Toolbox and a literature search of substances containing the hydroxycarboxylic acid functional group, which found only non-genotoxic substances.

Metabolite B was predicted to be non-genotoxic on the basis of modelling using both Derek Nexus and Leadscope and the lack of genotoxicity alerts of potential concern identified by the OECD QSAR Toolbox.

Toxicological evaluation

On the basis of in silico data, the Meeting concluded that metabolites A (acetochlor *tert*-sulfinyllactic acid) and B (acetochlor 1-hydroxyethyl *sec*-oxanilic acid) are unlikely to be genotoxic. Following the "Plant and animal metabolite assessment scheme", the Meeting concluded that, for chronic toxicity, these two metabolites could be assessed using the threshold of toxicological concern (TTC) approach. Both metabolites are categorized in Cramer class III, and therefore a TTC of 1.5 µg/kg bw per day applies.

The Meeting concluded that the information provided was insufficient to conclude definitively on the general toxicity of metabolites A and B relative to that of acetochlor.

References

EFSA (2016). Guidance on the establishment of the residue definition for dietary risk assessment. EFSA Panel on Plant Protection Products and their Residues (PPR). EFSA J. 14(2):4549, 129 pp. (https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2016.4549).

Pellizzaro M, Beevers C (2018). In silico genotoxicity assessment of *tert*-sulfinyllactic acid and 1-hydroxyethyl *sec*-oxanilic acid; two metabolites of acetochlor identified in soybean seed. Unpublished report. Exponent International Ltd Document No. 1806321.UKO-7681. Monsanto Report No. MSL0029932. Submitted to WHO by Bayer Crop Sciences.

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WHO (2015). Guidance document for WHO monographers and reviewers. Geneva: World Health Organization, WHO Core Assessment Group on Pesticide Residues (https://www.who.int/foodsafety/publications/JMPR-guidance-document/en/, accessed 8 May 2019).

BOSCALID (addendum)

First draft prepared by
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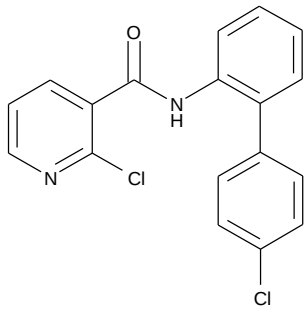
Explanation

Boscalid (2-chloro-*N*-[2-(4-chlorophenyl)phenyl]pyridine-3-carboxamide; Fig. 1) was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2006, when an acceptable daily intake (ADI) of 0–0.04 mg/kg body weight (bw) was established. The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for boscalid (Annex 1, reference *109*).

Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, boscalid was placed on the agenda of the present Meeting, which assessed additional toxicological information available on boscalid and its metabolites since the last review. The Meeting also applied the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015) for the assessment of these metabolites.

Fig. 1. Chemical structure of boscalid



Source: Stauer & Roth (2018)

The newly submitted studies investigated the absorption, excretion and metabolism, dermal sensitization, mechanism of the thyroid effects and reversibility of the toxicity of boscalid as well as the toxicity of M510F47, a low-level rat metabolite, a soil degradate and potentially a groundwater degradate, and M510F49, a soil degradate and potentially a groundwater degradate.

All critical studies contained statements of compliance with good laboratory practice and were conducted in accordance with relevant national or international test guidelines, unless otherwise specified. No additional information from a literature search was identified that complemented the toxicological information submitted for the current assessment.

Evaluation for acceptable intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

Four male and four female Wistar rats were treated with unlabelled boscalid (purity 95.7%; lot/batch no. N46) by gavage at a dose of 500 mg/kg bw per day for 14 or 28 consecutive days, followed by a single oral dose of [diphenyl-U-^{14}C]boscalid (purity >99%; lot/batch no. 641-2017) at 500 mg/kg bw. In the control group, four male and four female rats were treated orally with vehicle only (aqueous 0.5% carboxymethylcellulose containing 1% Cremophor EL) followed by a single dose of ^{14}C-labelled boscalid at 500 mg/kg bw. Urine and faeces were collected up to 48 hours after the treatment, and the samples for males and females of the different test groups were pooled.

Approximately 68–76% of the applied radioactivity was excreted in the faeces. In urine, the detected radioactivity was approximately 9–11% of the applied dose for males and approximately 14–18% for females. The excretion of radioactivity after a single oral administration of ^{14}C-labelled boscalid in each group is summarized in Table 1.

In conclusion, the toxicokinetics of boscalid following single or multiple oral administration to rats indicated no significant differences in the excretory patterns in urine and faeces, regardless of dosing regimen or sex (Fabian, Grosshans & Mellert, 2003).

(b) Dermal route

In vitro

The dermal penetration of boscalid formulated as BAS 510 01F (50% boscalid; wettable granule-type formulation) was assessed in vitro. The diffusion of ^{14}C-labelled boscalid (purity >98%; lot/batch no. 640-2301) into and through human skin was assessed by a single topical application of a target dose of 2500 or 3.85 µg/cm^2 (equivalent to a 1:1300 dilution) of test substance to split-thickness

Table 1. Excretion of radioactivity after a single oral administration of ^{14}C-labelled boscalid within 48 hours after dosing

	% of administered radioactivity					
Matrix	**Males (controls)**	**Females (controls)**	**Males (14 days[a])**	**Females (14 days[a])**	**Males (28 days[a])**	**Females (28 days[a])**
Urine	10.21	17.68	8.61	13.97	10.53	14.76
Faeces	75.54	71.55	75.65	69.19	67.67	69.84
Cage wash	0.53	0.97	0.55	0.42	0.51	0.91
Total	86.28	90.20	84.81	83.58	78.71	85.51

[a] Days of treatment with unlabelled boscalid.
Source: Fabian, Grosshans & Mellert (2003)

human skin preparations mounted on flow-through diffusion cells. The study was performed using 16 diffusion cells, all of which were assessed to be valid.

The mean recoveries and kinetic parameters of the dermal absorption of boscalid are shown in Table 2. The mean absorbed dose (receptor fluid only) was 0.05% and 1.03% of the applied dose for the high and low doses, respectively. Minor amounts of the test substance were associated with the skin and tape strips (excluding tape strips 1 and 2) after the exposure period. This amount accounted for 0.02% and 0.11% of the applied dose for the high and low doses, respectively.

Table 2. Mean recoveries and kinetic parameters of dermal absorption of boscalid

Parameter	**High dose**	**Low dose**
Target concentration (mg/mL)	250.0	0.385
Target dose of test substance (µg/cm^2)	2 500	3.85
Applied nominal dose of test substance (µg/cm^2)	3 090	3.62
Recovery (% of applied dose)		
Dislodgeable dose (donor chamber washing, skin washes)	95.07	97.39
Dose associated with tape strips		
Tape strip sample 1 (first two strips)	0.01	0.03
Tape strip sample 2 (remaining strips)	0.01	0.02
Dose associated with remaining skin		
Excluding tape strips	0.01	0.09
Absorbed dose (receptor fluid, receptor chamber washing)	0.05	1.03
Total recovery	95.15	98.56
Absorption kinetics		
Relative absorption within half study duration (%) (×10^{-5} cm/h)	0.05	0.04
Absorption rate (µg/(cm^2·h))	0.152	0.000 1
Lag time (h)	3.69	1.73

Source: Rieken, Fabian & Landsiedel (2017)

In conclusion, this dermal penetration study using human skin in vitro indicated that the dermal penetration estimates were 0.07% and 1% for the formulation concentrate (50% boscalid) and the 1:1300 spray dilution, respectively (Rieken, Fabian & Landsiedel, 2017).

1.2 Biotransformation

(a) In vivo

In the study by Fabian, Grosshans & Mellert (2003) described in section 1.1 above, the metabolite patterns in urine and faeces in each group were analysed by the application of two different high-performance liquid chromatography (HPLC) systems. Metabolites were identified by liquid chromatography (LC) with tandem mass spectrometry (MS/MS) and by their known retention time in the applied HPLC systems.

Identified metabolites in urine and faeces are shown in Table 3. Boscalid was the predominant compound in faeces, and M510F01 was the main metabolite in this matrix. The glucuronic acid conjugate M510F02 was not detected in any faecal extracts. The metabolite patterns in faeces of all test groups were comparable. In urine, there were no significant deviations between male and female animals, and the metabolic patterns were independent of the investigated test group.

Table 3. Identified metabolites of boscalid in urine and faeces of rats

Parent/ metabolite[a]	Composition of radioactive residues (% of dose)											
	Urine						Faeces					
	Controls		14 days[b]		28 days[b]		Controls		14 days[b]		28 days[b]	
	M	F	M	F	M	F	M	F	M	F	M	F
Boscalid	–	–	–	–	–	–	36.32	34.02	37.98	30.22	29.35	29.71
M510F02	2.78	14.33	4.29	9.55	5.56	10.75	–	–	–	–	–	–
M510F42	0.32	0.45	0.79	0.35	1.45	0.55	–	–	–	–	–	–
M510F03	1.63	0.17	1.07	0.16	1.18	0.14	–	–	–	–	–	–
M5I0F01	2.96	1.44	1.35	2.89	0.77	2.47	13.01	21.02	12.93	23.05	13.32	24.51
M510F20	0.45	–	0.31	0.17	0.27	0.19	4.20	2.80	7.19	3.27	8.32	4.88

[a] For the chemical structures of the metabolites of boscalid, the reader should refer to Annex 1, reference *109*.

[b] The number of days on which rats were treated orally with unradiolabelled boscalid. After the period of treatment with unradiolabelled boscalid, a single dose of radiolabelled boscalid was administered orally.

Source: Fabian, Grosshans & Mellert (2003)

In conclusion, analysis of metabolites following single or multiple oral administration of boscalid to rats indicated no significant differences in the metabolic patterns in urine and faeces, regardless of dosing regimen or sex (Fabian, Grosshans & Mellert, 2003).

(b) In vitro

An in vitro comparative metabolism study was performed with boscalid and human, rat and dog hepatocytes. The objective of the study was to determine whether the metabolic profiles were similar among species and whether unique human metabolites occurred. For this purpose, hepatocytes of humans, rats and dogs (mixed sexes for each species) were incubated with [pyridine-3-^{14}C]boscalid (chemical purity 98.0%; radiochemical purity 99.5%; lot/batch no. 640-2301; specific activity 5.11 MBq/mg) at a concentration of 10 µmol/L. After 0, 10, 30, 60 and 180 minutes of incubation, the samples were processed and analysed with HPLC. One replicate per triplicate was also analysed with HPLC-MS. HPLC-MS analyses were carried out to assign mass-to-charge ratio (*m*/*z*) values to those

peaks that accounted for more than 5% of the applied radioactivity in samples of human hepatocytes (for at least one time point). The viability of hepatocytes after incubation with boscalid at 10 µmol/L for 180 minutes was proven for all species using a luminescent cell viability assay.

The recovery was generally greater than 90% of the applied radioactivity, or only slightly lower, for all species.

The unchanged parent compound was detected in samples of hepatocytes from all species. For human hepatocytes, peaks at retention times of approximately 7.1 minutes (metabolite P7.1) and 14.9 minutes (metabolite P14.9) and metabolite M510F54 were relevant (>5% of the applied radioactivity for at least one time point). Metabolite P7.1 was also detected in samples of rat and dog hepatocytes, and metabolite P14.9 was also detected in rat hepatocytes. M510F54, a phase II metabolite, was specific to human and dog hepatocytes at comparable levels. Although M510F54 could not be identified in rat hepatocytes under the conditions of this study, additional information from an in vivo metabolism study (Grosshans & Knoell, 2000) provides evidence that this type of metabolism is also occurring in the rat, as shown by the formation of metabolite M510F03. M510F03 had already been identified in the metabolism pathway of boscalid in rats by the previous Meeting (Annex 1, reference *109*).

The results indicated that hydroxylation of the parent biphenyl group, followed by sulfation of the hydroxyl moiety to result in M510F54, was a common metabolic pathway in all three test species. No human-specific metabolites of boscalid were identified.

Detailed results are shown in Table 4. The chemical structure of M510F54 is shown in Fig. 2 (Funk & Lutz, 2016).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Single oral gavage doses of boscalid (purity 94.4%; lot/batch no. N37) in 0.5% aqueous carboxymethylcellulose solution at 1000 or 2000 mg/kg bw were administered to each of three male and three female Wistar rats. The respective control groups received only the vehicle (0.5% aqueous carboxymethylcellulose solution) at the dosing volume of 20 mL/kg bw. Mortality and general clinical signs were recorded, and detailed examination for clinical signs was performed immediately after test substance administration and 15 minutes, 30 minutes and hourly up to 6 hours after administration of the test substance. On days 1–3, the detailed examination was performed once daily. On day 6, the animals were euthanized without further examination.

No mortality occurred, and no clinical signs were observed in any animal of any dose group throughout the study period.

In conclusion, the median lethal dose (LD_{50}) was greater than 2000 mg/kg bw (Mellert, 2002).

(b) Dermal sensitization

Boscalid (purity 94.3%; batch no. N46) was tested in a maximization test in Dunkin-Hartley guinea-pigs according to the Magnusson and Kligman procedure.

The first challenge resulted in discrete or patchy erythema in 3/20 animals 24 hours after patch removal, which was completely reversible within the 48-hour reading time point. After the second challenge, discrete or patchy erythema was noted in 2/20 animals at 24 hours after patch removal, which was reversible in one of these animals and persisted in another animal until the 48-hour reading time point. The skin reactions observed in a few animals of the treated group after the challenges were considered to represent signs of slight irritation.

Table 4. Comparison of the relevant metabolites of boscalid after incubation with human, rat and dog hepatocytes

Incubation time (min)	Species	Range of recovery (% AR) in sample supernatants	Relevant peak (% of applied radioactivity)							
			Boscalid[a]	M510F54[b]	P7.1[c]	P10.6	P13.1	P14.9	P15.1	P17.5
0	Human	88–95	91.38	–	–	–	–	–	–	–
	Rat	87–89	88.13	–	–	–	–	–	–	–
	Dog	83–97	91.32	–	–	–	–	–	–	–
10	Human	97–100	98.14	–	–	–	–	–	–	–
	Rat	89–91	60.29	–	–	–	29.59	–	–	1.52
	Dog	85–98	63.18	–	10.83	–	–	–	–	18.32
30	Human	100–102	97.41	–	–	–	–	–	–	3.86
	Rat	91–94	30.31	–	4.53	–	56.99	–	–	–
	Dog	84–100	41.48	3.50	34.25	–	–	–	–	14.55
60	Human	96–99	80.54	4.73	5.66	2.70	3.20	–	–	4.11
	Rat	92–94	9.03	–	9.23	–	73.60	3.57	–	–
	Dog	94–96	6.42	6.20	78.43	–	4.52	–	–	2.58
180	Human	86–96	55.50	9.65	13.49	–	2.79	7.08	2.00	2.06
	Rat	87–91	–	–	11.06	–	68.81	7.65	2.31	–
	Dog	90–95	–	7.37	85.69	–	–	–	–	–

AR: applied radioactivity

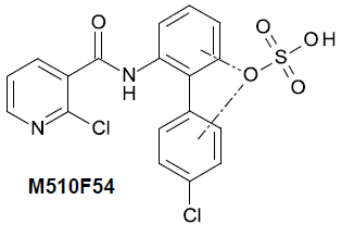
[a] The peaks at approximately 23.7–23.8 minutes were assigned to the parent compound, boscalid.

[b] The peaks at approximately 11.6–11.8 minutes were assigned to metabolite M510F54.

[c] P + the mean value of the corresponding retention time (e.g. P17.5 for peaks with a mean retention time of 17.5 minutes).

Source: Funk & Lutz (2016)

Fig. 2. Chemical structure of M510F54



Source: Funk & Lutz (2016)

Boscalid was not considered to be sensitizing to the skin of the guinea-pig in the maximization test (Gamer & Leibold, 2003).

2.2 Short-term studies of toxicity

(a) Exposure by inhalation

Groups of 10 male and 10 female Wistar rats were exposed to boscalid via nose-only exposure for 6 hours per day, 5 days per week, for 4 weeks (20 exposures). The target and analytically determined boscalid concentrations were 25, 125 and 625 mg/m^3 as a dust aerosol and 25.3, 125.1 and 616.7 mg/m^3, respectively. As the test substance strongly agglomerated, a dust atmosphere could not be generated with the unchanged test substance. To enable dust generation, 1% (by weight) Aerosil R 972 (an amorphous silicate often used as an anticaking agent) was added to the test substance. Thus, a vehicle control group consisting of 10 male and 10 female rats was exposed to Aerosil R 972 at 6 mg/m^3.

The diameters of particles in the vehicle, 25.3 mg/m^3, 125.1 mg/m^3 and 616.7 mg/m^3 groups were 0.0–0.7 µm ± 8.6–19.3, 1.7–2.0 µm ± 2.5–2.6, 1.8–2.2 µm ± 2.1–2.3 and 2.2–3.7 µm ± 2.3–3.1, respectively, expressed as mass median aerodynamic diameter ± geometric standard deviation.

Aerosil R 972, the vehicle control substance, induced a slight inflammatory reaction, with findings of neutrophilia in blood, increased lung weights, multifocal mixed cell inflammation in the lungs, macrophage aggregation in the bronchus-associated lymphoid tissue and mediastinal and tracheobronchial lymph nodes, and lymphoreticulocyte hyperplasia in the mediastinal and tracheobronchial lymph nodes in both sexes. Similar findings, with lower incidence and severity, were observed in male and female rats exposed to boscalid at 125.1 and 616.7 mg/m^3. No findings indicating systemic toxicity of boscalid were observed. Thus, the microscopic findings in the lungs and mediastinal and tracheobronchial lymph nodes in boscalid-treated groups were considered to be due to the vehicle, Aerosil R 972.

The no-observed-adverse-effect concentration (NOAEC) of boscalid for nose-only inhalation toxicity in rats was 616.7 mg/m^3, the highest measured concentration (Ma-Hock et al., 2017).

2.3 Special studies

(a) Mechanistic studies

Induction of metabolizing enzymes in the liver and changes in thyroid hormone levels

To investigate the induction of metabolizing enzymes in the liver and changes in thyroid hormone levels in rats, boscalid (purity 95.7%; lot/batch no. N46) was administered to groups of 10 male and 10 female Wistar rats at a dietary concentration of 0, 500, 2000 or 5000 parts per million (ppm) (equal to 0, 29.6, 117.3 and 294.2 mg/kg bw per day for males and 0, 34.6, 141.9 and 355.3 mg/kg bw per day for females, respectively) for 4 weeks. Feed consumption and body weights were determined weekly, and the animals were examined for signs of toxicity or mortality at least once a day. Levels of thyroid hormones (triiodothyronine [T_3], thyroxine [T_4] and thyroid stimulating hormone [TSH]) in the serum were determined on days 7, 14 and 28. At the end of the study, liver and thyroid were weighed, and the activities of several phase I and phase II enzymes were determined using liver microsomes.

No mortality or clinical signs were observed during the study period. Treatment with the test substance had no effect on feed consumption or body weight. Decreased T_4 levels and increased TSH levels were observed in males of the mid- and high-dose groups. In the high-dose females, TSH levels were consistently, but not statistically significantly, increased (18–32%). Increased absolute liver (up to 32%) and thyroid (up to 46.7%) weights were observed in treated animals of both sexes at all doses. Similar results were observed for relative liver and thyroid weights. In general, the liver effect was more pronounced in male animals. Most of the phase I and phase II enzyme activities were increased in animals of both sexes at the middle and high doses.

This study indicated that treatment with boscalid led to decreases in T_4 levels, increases in TSH levels, increased liver and thyroid weights and increased activities of phase I and phase II enzymes. It

can be concluded that the mild imbalance in thyroid hormone levels caused by boscalid was due to the induction of the hepatic microsomal enzyme system (Mellert et al., 2003).

Reversibility of repeated-dose toxicity in rats

To examine the reversibility of effects following repeated dosing, boscalid (purity 94.1%; lot/batch no. N46) was administered to groups of five male Wistar rats at a dietary concentration of 0, 100, 2500 or 15 000 ppm (equal to 0, 7.7, 190.3 and 1137.4 mg/kg bw per day, respectively) for 4 weeks. The administration period was followed by a treatment-free recovery period of 0, 28 or 91 days. Feed consumption and body weights were determined weekly, and the animals were examined for signs of toxicity or mortality at least once a day. Levels of thyroid hormones (T_3, T_4, TSH) in the serum were measured before the treatment, at the end of dosing and at the end of the recovery period. All animals were subjected to gross pathological examination, followed by organ weight measurements as well as histopathological evaluation of liver and thyroid.

No treatment-related changes in mortality, clinical signs, feed consumption or body weight were observed. Increased liver weights with centrilobular hypertrophy of hepatocytes (zone 3) and portal (zone 1) fatty change were observed at the end of the administration period at 2500 ppm and above. No effects on T_3 or T_4 levels were observed at any dose. Increased TSH levels, increased thyroid weight and associated hypertrophy and diffuse hyperplasia of follicular cells were observed at 2500 ppm and above. These changes were reversible within 4 weeks after the end of the administration period (Mellert et al., 2001).

Mechanism of effect of boscalid on thyroid in rats

To investigate the mechanism of the effect of boscalid on the thyroid, boscalid (purity 95.7%; lot/batch no. N46) at 5000 ppm, propylthiouracil at 2000 ppm or phenobarbital at 1000 ppm was administered to male Wistar rats (12 per group) in the diet for 2 weeks. On study day 14, the rats were administered radiolabelled (^{125}I) sodium iodide intraperitoneally; 6 hours later, half of the rats in each group were administered potassium perchlorate in 0.9% saline solution at 10 mg/kg bw intraperitoneally, and half were administered 0.9% saline solution at 10 mL/kg bw intraperitoneally. Two and a half minutes after the treatment with potassium perchlorate or saline solution, the animals were examined.

Thyroid weights were increased by boscalid treatment (not statistically significant) and by phenobarbital and propylthiouracil treatments (statistically significant). ^{125}I uptake in the thyroid was statistically significantly higher following boscalid and phenobarbital treatments compared with the concurrent controls, but no statistically significant discharge of ^{125}I occurred after perchlorate administration. ^{125}I uptake in the thyroid was markedly decreased by propylthiouracil treatment compared with the concurrent controls, and a statistically significant discharge of ^{125}I occurred after perchlorate administration.

The results indicated an indirect effect of boscalid on the thyroid, similar to that of phenobarbital (Cunha, Deckadt & Van Ravenzwaay, 2004).

3. Toxicological data on metabolites and/or degradates

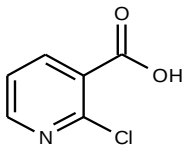
The chemical structures of M510F47, a low-level rat metabolite, a soil degradate and potentially a groundwater degradate, and M510F49, a soil degradate and potentially a groundwater degradate, are shown in Fig. 3.

Toxicity studies on M510F47 and M510F49 were submitted to the present Meeting, including acute oral toxicity studies (M510F47 and M510F49), a 90-day dietary toxicity study (M510F49 only) and genotoxicity studies in vitro (M510F47 and M510F49) and in vivo (M510F49 only). Results of the acute toxicity and genotoxicity studies are summarized in Tables 5 and 6, respectively.

Fig. 3. Chemical structures of M510F47 and M510F49

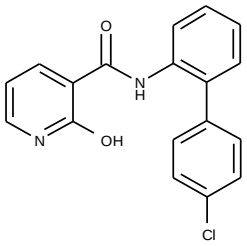
M510F47

2-Chloronicotinic acid



M510F49

N-(4′-Chlorobiphenyl-2-yl)-2-hydroxynicotinamide



Source: Stauber & Roth (2019)

Table 5. Summary of acute toxicity of M510F47 and M510F49

Metabolite/ degradate	Species/strain	Sex	Route	Purity/batch no.	LD_{50}	Reference
M510F47	Rat/Wistar	M/F	Oral	99.8% / 01174-232	>2 000 mg/kg bw	Kuehlem & Hellwig (1998)
M510F49	Rat/Wistar	M/F	Oral	99.7% / 01742-59	>2 000 mg/kg bw	Gamer & Hoffmann (2001)

bw: body weight; F: females; LD_{50}: median lethal dose; M: males

Table 6. Summary of genotoxicity of M510F47 and M510F49

End-point	Test object	Concentrations/doses	Purity/ lot no.	Results	Reference
M510F47					
In vitro					
Reverse mutation assay	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) *Escherichia coli* (WP2 *uvr*A)	SPT: 20–5 000 µg/plate with/without S9 PIT: 125–2 000 µg/plate with/without S9	99.8% / 01174-232	Negative	Engelhardt& Hoffmann (1998)
Gene mutation test, *Hprt* locus	CHO cells	Exp. 1: 0, 200.0, 400.0, 800.0 and 1 600.0 µg/mL with/without S9 Exp. 2: 0, 250.0, 500.0, 1 000.0 and 1 600.0 µg/mL with/without S9 Exp. 3: 0, 250.0, 500.0, 1 000.0 and 1 600.0 µg/mL with S9	100% / L80-188	Negative	Schulz & Landsiedel (2015a)
Micronucleus assay	Chinese hamster V79 cells	Exp. 1: 0, 49.4, 98.8, 197.5, 395.0, 790.0 and 1 580.0 µg/mL with/without S9	100% / L80-188	Negative	Schulz & Landsiedel (2015b)

End-point	Test object	Concentrations/doses	Purity/ lot no.	Results	Reference
		Exp. 2: 0, 98.8, 197.5, 395.0, 790.0 and 1 580.0 µg/mL with/without S9			
M510F49					
In vitro					
Reverse mutation assay	*S. typhimurium* (TA98, TA100, TA1535, TA1537) *E. coli* (WP2 *uvr*A)	SPT: 0, 20, 100, 500, 2 500 and 5 000 µg/plate with/without S9 PIT: 0, 4, 20, 100, 500 and 2 000 µg/plate with/without S9	99.7% / 01742-59	Negative	Engelhardt & Hoffmann (2000)
Gene mutation test, *Hprt* locus	CHO cells	Exp. 1: 6.3, 12.5, 25, 50 and 100 µg/mL with/without S9 Exp. 2: 12.5, 25, 50, 100, 412.5, 825, 1 650 and 3 300 µg/mL without S9; 10, 20, 40, 80 and 100 µg/mL with S9	99.7% / L71-12	Negative	Schulz & Landsiedel (2014)
In vivo					
Micronucleus assay	Bone marrow of NMRI mice	Twice a day at a dose of 0, 500, 1 000 or 2 000 mg/kg bw intraperitoneally	98.9% / 01196-217	Negative	Engelhardt & Hoffmann (2001)

bw: body weight; CHO: Chinese hamster ovary; Exp.: experiment; *Hprt*: hypoxanthine–guanine phosphoribosyltransferase; PIT: preincubation test; S9: 9000 × *g* supernatant fraction from rat liver homogenate; SPT: standard plate test

3.1 M510F47 (Reg. No. 107371, 2-chloronicotinic acid)

(a) Acute toxicity

Male and female Wistar (Chbb:Thom (SPF)) rats were administered M510F47 (purity 99.8%; batch no. 01174-232) as a single dose of 2000 mg/kg bw in an aqueous suspension (dosing volume of 10 mL/kg bw) by gavage and were observed for a period of 14 days. Gross necropsy was performed at the end of the study period (day 14).

No mortalities occurred, and no signs of toxicity were observed during the study period. Body weight and body weight gain were unaffected by the test item. At necropsy, no pathological changes were observed that could be attributed to administration of M510F47.

The acute oral LD_{50} of M510F47 was greater than 2000 mg/kg bw (Kuehlem & Hellwig, 1998).

(b) Genotoxicity

In the reverse mutation assay, M510F47 was tested via the standard plate test and the preincubation test in concentrations ranging from 20 to 5000 µg/plate and from 125 to 2000 µg/plate, respectively. Precipitation of the test item was observed at 5000 µg/plate. Bacteriotoxicity of the test item was observed at concentrations of 2500 µg/plate and above in the standard plate test and of 1000 µg/plate and above in the preincubation test. An increase in the number of his^+ and trp^+ revertants was not observed in the standard plate test or the preincubation test either with or without metabolic activation (S9). The positive controls induced the appropriate response (Engelhardt & Hoffmann, 1998).

In a test for gene mutations using the *Hprt* locus assay in Chinese hamster ovary (CHO) cells, the first experiment was conducted at concentrations of 0, 200.0, 400.0, 800.0 and 1600.0 µg/mL with or without S9 for a 4-hour exposure period; the second experiment was conducted at 0, 250.0, 500.0,

1000.0 and 1600.0 µg/mL with or without S9 for a 4-hour exposure period; and the third experiment was conducted at 0, 250.0, 500.0, 1000.0 and 1600.0 µg/mL with S9 for a 4-hour exposure period. Both positive control substances, ethyl methanesulfonate (EMS) and 7,12-dimethylbenz[*a*]anthracene (DMBA), led to the expected increase in the frequencies of forward mutations, except in the second experiment with S9. Therefore, this experimental part was repeated and designated as the third experiment. In this study, in the absence and presence of metabolic activation, no cytotoxicity was observed up to the highest required concentration evaluated for gene mutations. Results indicated that M510F47 did not cause any biologically relevant increase in the mutant frequencies in three experiments performed independently of each other. Thus, under the experimental conditions of this in vitro study, M510F47 was not mutagenic in the *Hprt* locus assay in CHO cells (Schulz & Landsiedel, 2015a).

In an in vitro micronucleus assay using Chinese hamster V79 cells, the first experiment was conducted at concentrations of 0, 49.4, 98.8, 197.5, 395.0, 790.0 and 1580.0 µg/mL with or without S9 for 4 hours, and the second experiment was conducted at 0, 98.8, 197.5, 395.0, 790.0 and 1580.0 µg/mL without S9 for 24 hours or with S9 for 4 hours. A sample of at least 1000 cells for each culture was analysed for micronuclei (i.e. 2000 cells for each test group). The vehicle controls gave frequencies of micronucleated cells within the laboratory historical negative control data range for V79 cells. Both positive control substances, EMS and cyclophosphamide, led to the expected increase in the number of cells containing micronuclei. In this study, no cytotoxicity, indicated by a reduced cytokinesis-block proliferation index or cell numbers, was observed up to the highest required test substance concentration. The statistically significant increase in cells containing micronuclei obtained in the second experiment in the presence of metabolic activation occurred at one intermediate dose only, and this finding was not considered to be biologically relevant. Results indicated that M510F47 did not cause any biologically relevant increase in the number of cells containing micronuclei either with or without S9. Thus, under the experimental conditions described, M510F47 was not considered to have a chromosome-damaging (clastogenic) effect or to induce numerical chromosomal aberrations (aneugenic activity) in V79 cells in vitro in the absence or presence of metabolic activation (Schulz & Landsiedel, 2015b).

3.2 M510F49 (Reg. No. 391572, N-(4'-chlorobiphenyl-2-yl)-2-hydroxynicotinamide)

(a) Acute toxicity

A single dose of M510F49 (purity 99.7%; batch no. 01742-59) suspended in olive oil at 2000 mg/kg bw was given to three fasted male and female Wistar (Crl:WI (GLX/BRL/HAN)IGS BR) rats by gavage. The animals were observed for 14 days.

No mortality occurred in the study. No clinical signs or findings were observed. The mean body weights of the treated groups increased throughout the study period. No macroscopic pathological abnormalities were noted in the animals examined at the end of the observation period.

The acute oral LD_{50} of M510F49 was greater than 2000 mg/kg bw (Gamer & Hoffmann, 2001).

(b) Short-term studies of toxicity

In a short-term study, M510F49 (purity 99.2%; batch no. L85-64) was administered to Crl:WI(Han) rats (10 animals of each sex per group) at a dietary concentration of 0, 150, 1500 or 15 000 ppm (equal to 0, 9.7, 95 and 968 mg/kg bw per day for males and 0, 11, 110 and 1082 mg/kg bw per day for females, respectively) for at least 90 days.

No animal died during the study period. No treatment-related effects on clinical signs, feed consumption, functional observational battery/motor activity, ophthalmoscopy, clinical pathology or pathological analysis were observed. The body weight gains of male rats at 1500 and 15 000 ppm were slightly (approximately 5–8%) decreased during the study; however, these decreases were not statistically significantly different from control values. Owing to their small magnitude and the absence

of statistical significance, these changes in body weight gain were not considered to be toxicologically relevant.

The no-observed-adverse-effect level (NOAEL) for M510F49 in a 90-day feeding study in rats was 15 000 ppm (equal to 968 mg/kg bw per day), the highest dose tested (Buesen, 2015).

(c) Genotoxicity

In a reverse mutation assay (standard plate test and preincubation test), M510F49 was treated at concentrations of 20–5000 µg/plate with and without S9. In the preincubation test, the test item was tested at concentrations of 4–2000 µg/plate, also with and without metabolic activation. Precipitation of M510F79 was found from about 500 µg/plate onward. A slight decrease in the number of revertants was occasionally observed, depending on the strain and test conditions, from about 2000–2500 µg/plate onward. An increase in the number of his^+ or trp^+ revertants was not observed in either the standard plate test or the preincubation test, either with or without S9. The positive controls induced the appropriate response. According to the results of the study, M510F79 was not mutagenic in the Ames standard plate or preincubation test (Engelhardt & Hoffmann, 2000).

A gene mutation test was conducted to clarify the in vitro ability of M510F49 to induce forward mutations in mammalian cells by assessing the mutation of the *Hprt* locus in CHO cells. Two independent experiments were conducted in the presence and absence of metabolic activation. CHO cells were incubated with M510F49 at a dose of 6.3, 12.5, 25, 50 or 100 µg/mL for 4 hours with or without S9 in experiment 1 and at a dose of 12.5, 25, 50, 100, 412.5, 825, 1650 or 3300 µg/mL for 24 hours without S9 and 10, 20, 40, 80 or 100 µg/mL for 4 hours with S9. EMS and DMBA served as positive controls in the experiments. No cytotoxic effects were observed in any experiment under all test conditions up to the highest applied concentration. In the second experiment, in the absence of metabolic activation after 24 hours of exposure, the cell densities were distinctly reduced in the first experiment at 50 and 100 µg/mL. These concentrations were close to the limit of test substance solubility in culture medium. In both test groups, the cloning efficiencies were only weakly affected. Cell morphology was not influenced by treatment. The test substance did not cause any relevant increase in the mutant frequencies either with or without S9 in two experiments performed independently. Based on the results of the study, it was concluded that under the conditions of this test, M510F49 did not induce forward mutations in mammalian cells in vitro (Schulz & Landsiedel, 2014).

In a micronucleus test to investigate chromosomal damage (clastogenicity/aneugenicity) in NMRI mice (five mice of each sex per group for the range-finding study and five male mice per group for the main study), M510F49 suspended in 0.5% carboxymethylcellulose was administered twice (within 24 hours) intraperitoneally to mice at a dose of 500, 1000 or 2000 mg/kg bw in a dosing volume of 20 mL/kg bw. The vehicle served as a negative control, and cyclophosphamide and vincristine served as positive controls. The animals were euthanized 24 hours after the second administration, and the bone marrow of the two femora was prepared from each animal. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The number of normochromatic erythrocytes occurring per 2000 polychromatic erythrocytes was also recorded. The administration of M510F49 did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing micronuclei. The rate of micronucleus formation was generally close to that of the concurrent negative control and was within the range of the historical control data. A slight inhibition of erythropoiesis was occasionally detected in individual animals of the 1000 and 2000 mg/kg bw dose groups. Clinical signs observed at 1000 and 2000 mg/kg bw included poor general state and squatting posture, which were fully reversible within 48 hours after dose administration. No signs of systemic toxicity were observed in any of the animals treated with the positive control substances or the vehicle. The positive control chemicals cyclophosphamide and vincristine led to the expected increase in the polychromatic erythrocytes containing micronuclei. Thus, under the experimental conditions of this study, M510F49 did not induce cytogenetic damage (clastogenic or aneugenic) in bone marrow cells of NMRI mice in vivo (Engelhardt & Hoffmann, 2001).

4. Observations in humans

4.1 Medical surveillance of manufacturing plant personnel

All persons handling crop protection products are subject to regular medical examinations. There are no specific parameters available for monitoring the effects of boscalid. Thus, the medical monitoring programme is designed as a general health checkup, with special interest in the primary target organs presumed to be relevant in humans by analogy with the results of animal experiments.

The surveillance programme includes a general physical examination, including neurological status, red and white blood cell counts and liver enzymes. No adverse health effects suspected to be related to boscalid exposure have been observed (Stauber & Roth, 2018).

4.2 Clinical cases and poisoning incidents

One case of slight skin irritation has been registered in the sponsor's internal clinical incident log for employees accidentally exposed to boscalid in combination with another product, and it is not clear whether boscalid was the cause of this irritation.

No data on exposure of the general public or epidemiological studies are available from the sponsor, nor is the sponsor aware of any epidemiological studies performed by third parties (Stauber & Roth, 2018).

Comments

Biochemical aspects

The toxicokinetics of boscalid in rats following the administration of a single dose of ^{14}C-labelled boscalid at 500 mg/kg bw or multiple dosing of unlabelled boscalid at 500 mg/kg bw per day for 14 or 28 days followed by a single dose of ^{14}C-labelled boscalid at 500 mg/kg bw indicated no significant differences in the excretory (approximately 70% in the faeces) or metabolic patterns in urine and faeces, regardless of dosing regimen or sex (Fabian, Grosshans & Mellert, 2003).

In dermal penetration studies using human skin in vitro, the dermal penetration estimates were 0.07% and 1% for the formulation concentrate (50% boscalid) and the 1:1300 spray dilution, respectively (Rieken, Fabian & Landsiedel, 2017).

In an in vitro study comparing the metabolism of boscalid in human, rat and dog hepatocytes, no human-specific metabolites of boscalid were identified, and the metabolic pathways were similar in the tested species (Funk & Lutz, 2016).

Toxicological data

In rats, the acute oral LD_{50} for boscalid was greater than 2000 mg/kg bw (Mellert, 2002). Boscalid was not sensitizing to the skin of guinea-pigs (Gamer & Leibold, 2003).

In a study to investigate the induction of metabolizing enzymes in the liver and changes in thyroid hormone levels in rats, treatment with boscalid led to decreases in T_4 levels, increases in TSH levels, increased liver and thyroid weights and increased activities of phase I and phase II enzymes. It was concluded that the mild imbalance in thyroid hormone levels caused by boscalid was due to the induction of the hepatic microsomal enzyme system (Mellert et al., 2003). The effects of boscalid on liver and thyroid were reversible (Mellert et al., 2001), and the effect on the thyroid was considered indirect (Cunha, Deckardt & Van Ravenzwaay, 2004).

Toxicological data on metabolites and/or degradates

M510F47 (Reg. No. 107371, 2-chloronicotinic acid; low-level rat metabolite, soil and potentially groundwater degradate)

M510F47 had low acute toxicity (LD_{50} > 2000 mg/kg bw; Kuehlem & Hellwig, 1998) and showed no evidence of genotoxicity in vitro (Engelhardt & Hoffmann, 1998; Schulz & Landsiedel, 2015a,b).

M510F49 (Reg. No. 391572, N-(4'-chlorobiphenyl-2-yl)-2-hydroxynicotinamide; soil and potentially groundwater degradate)

M510F49 had low acute toxicity (LD_{50} > 2000 mg/kg bw; Gamer & Hoffmann, 2001) and showed no evidence of genotoxicity in vitro or in vivo (Engelhardt & Hoffmann, 2000, 2001; Schulz & Landsiedel, 2014).

The NOAEL for M510F49 identified in a 90-day feeding study in rats was 15 000 ppm (equal to 968 mg/kg bw per day), the highest dose tested (Buesen, 2015). The Meeting noted that the NOAELs for boscalid in 90-day feeding studies were 34 mg/kg bw per day in rats, 29 mg/kg bw per day in mice and 7.6 mg/kg bw per day in dogs, as identified by the 2006 Meeting (Annex 1, reference *109*).

Human data

No adverse effects of boscalid were reported in medical surveillance of manufacturing plant personnel. One case of slight skin irritation was registered in an employee accidentally exposed to boscalid in combination with another product. Therefore, it was not clear whether the effect was attributable to boscalid (Stauber & Roth, 2018). No data on exposure of the general public or epidemiological studies are available for boscalid.

Toxicological evaluation

The Meeting concluded that it was not necessary to revise the ADI or establish an ARfD for boscalid.

The Meeting concluded that metabolite M510F47 was unlikely to be genotoxic. Following JMPR's "Plant and animal metabolite assessment scheme" (WHO, 2015), the Meeting concluded that for chronic toxicity, M510F47 could be assessed using the threshold of toxicological concern (TTC) approach. M510F47 is categorized in Cramer class III, and therefore a TTC of 1.5 µg/kg bw per day applies.

On the basis of a comparison of NOAELs in short-term studies of toxicity, the Meeting concluded that the toxicity of M510F49 was lower than that of the parent compound. Owing to the limited database on M510F49, the Meeting was unable to conclude that this degradate was of no concern, but concluded that it would be covered by the ADI of the parent compound.

The ADI of 0–0.04 mg/kg bw applies to boscalid plus M510F49, expressed as boscalid.

Acceptable daily intake (ADI) (applies to boscalid plus M510F49, expressed as boscalid)

0–0.04 mg/kg bw

Critical end-points for setting guidance values for exposure to boscalid and metabolites/degradates

Acute toxicity	
Rat, LD_{50}, oral	>2 000 mg/kg bw
Dermal sensitization (guinea-pig)	Not sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	None
Lowest relevant inhalation NOAEC	616.7 mg/m^3, highest concentration tested (rat)
Studies on metabolites/degradates	
M510F47	Oral LD_{50} > 2 000 mg/kg bw
	No evidence of genotoxicity in vitro
M510F49	Oral LD_{50} > 2 000 mg/kg bw
	No evidence of genotoxicity
	Short-term toxicity NOAEL: 968 mg/kg bw per day, highest dose tested (rat)

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CHLOROTHALONIL (addendum)

First draft prepared by
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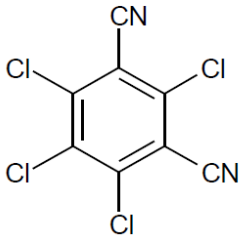
Explanation

Chlorothalonil (tetrachloroisophthalonitrile) (Fig. 1) was previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2009, when an acceptable daily intake (ADI) of 0–0.02 mg/kg body weight (bw) and an acute reference dose (ARfD) of 0.6 mg/kg bw were established. The 2009 Meeting also established an ADI of 0–0.008 mg/kg bw and an ARfD of 0.03 mg/kg bw for metabolite SDS-3701 (2,5,6-trichloro-4-hydroxyisophthalonitrile) (Annex 1, reference *118*). In 2010,

the Meeting evaluated metabolite R611965 (3-carbamyl-2,4,5-trichlorobenzoic acid; SDS-46851) and considered it unnecessary to establish a separate ADI and ARfD for R611965 because of its lower toxicity in comparison with the parent compound (Annex 1, reference *121*).

Fig. 1. Structure of chlorothalonil



Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, chlorothalonil was placed on the agenda of the present Meeting, which assessed additional toxicological information available since the last review.

The new information on chlorothalonil included studies on absorption, distribution, metabolism and excretion, inhalation toxicity, acute and 90-day neurotoxicity, immunotoxicity, endocrine activity and phototoxicity. For six chlorothalonil metabolites and/or degradates, the new information included studies on acute toxicity, short-term toxicity and genotoxicity.

All critical studies contained statements of compliance with good laboratory practice (GLP) and were conducted in accordance with relevant national or international test guidelines, unless otherwise specified. No additional information from a literature search was identified that complemented the toxicological information submitted for the current assessment.

Evaluation for acceptable intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

In a repeated-dose study, ^{14}C-labelled chlorothalonil (purity >98.0%) was administered to seven female Wistar rats by gavage in 14 consecutive daily doses of 1.5 mg/kg bw. One animal was euthanized at each of four different time points during the dosing period (days 2, 5, 8 and 11) and three different time points after the last dosing (days 15, 17 and 21). The [^{14}C]chlorothalonil-related residues in tissues and organs were determined by means of a quantitative whole-body autoradiography technique. The excreted radioactivity was determined in urine and faeces of three animals at daily intervals.

[^{14}C]Chlorothalonil was poorly absorbed from the gastrointestinal tract into the systemic circulation. A steady state in terms of excretion was reached within 2 days after the start of dosing. About 80% of the daily dose was excreted in faeces after 2 days and until the end of dosing, whereas only 7% of the daily dose was excreted in urine. After the dosing period, the daily excretion decreased rapidly.

The blood kinetics showed increasing concentrations of radioactivity with ongoing administration. A steady state was achieved 12 days after the start of dosing, at a plateau level of about 0.050–0.060 µg chlorothalonil equivalents (equiv) per gram. After the last of 14 consecutive daily doses, the concentration in whole blood decreased slowly. The terminal half-life in blood was calculated to be 89 hours.

The highest concentrations were found in organs involved in the elimination process (i.e. kidney and liver). The highest residue level was found in the kidney cortex 24 hours after the last dosing, accounting for 2.453 µg equiv/g. Besides these, quantifiable residues were also found in thyroid, lung, blood, adrenals and ovaries. In all tissues and organs in which radioactivity could be determined, the

steady state was achieved within 5 days. After the dosing period, the residues in tissues and organs, if quantifiable, depleted with half-lives of 2–10 days (Hassler, 2007).

In another study, the pharmacokinetics, absorption, metabolism and excretion of total radioactivity were investigated following a single oral gavage administration of [^{14}C]chlorothalonil (purity 99.2%) at 5 or 200 mg/kg bw in 0.5% aqueous carboxymethylcellulose to a group of six male and six female Han Wistar rats. Blood and plasma were collected at intervals up to 3 days post-dosing to characterize the pharmacokinetics of total radioactivity. To characterize the absorption and excretion of total radioactivity, urine and faeces were collected at intervals up to 7 days post-dosing. In a separate study, bile duct–cannulated rats (three of each sex) were administered a single gavage dose of chlorothalonil at 5 or 200 mg/kg bw, and bile, urine and faeces were collected for up to 72 hours.

Following a single 5 mg/kg bw dose of [^{14}C]chlorothalonil to male and female rats, radioactivity was detectable in blood from the first time point (0.5 hour). The maximum concentration (C_{max}) of radioactivity in blood was 0.21 µg equiv/g in males and 0.31 µg equiv/g in females, with a time to reach C_{max} (T_{max}) of 8 hours post-dosing in males and 4 hours post-dosing in females. Concentrations in males declined rapidly between 12 and 24 hours, then gradually to 54 hours post-dosing. Concentrations in females maintained a plateau between 8 and 12 hours post-dosing, before declining rapidly at 24 hours post-dosing. Concentrations then decreased steadily to 54 hours post-dosing. Radioactivity was still detected in blood in both male and female rats at 54 hours post-dosing. Concentrations of radioactivity were detectable in plasma from the first time point (0.5 hour). The C_{max} values in plasma were higher than those in blood, at 0.52 and 0.58 µg equiv/g in males and females, respectively. The T_{max} in plasma occurred at 8 hours post-dosing in both males and females, after which concentrations decreased steadily up to 72 hours post-dosing. Radioactivity was still detected in plasma in both male and female rats at 72 hours post-dosing.

Following a single 200 mg/kg bw dose of [^{14}C]chlorothalonil to male and female rats, radioactivity was detectable in blood from the first time point (0.5 hour). The C_{max} values in blood were 3.2 and 6.0 µg equiv/g in males and females, respectively, with a T_{max} of 12 hours post-dosing in both males and females. Concentrations in males declined steadily until 54 hours post-dosing and fell below the limit of detection by 72 hours post-dosing. Concentrations in females also declined steadily, but fell below the limit of detection by 48 hours post-dosing. Radioactivity was detectable in plasma from the first time point (0.5 hour). The C_{max} values in plasma were higher than those in blood, at 6.8 and 11.6 µg equiv/g in males and females, respectively. The T_{max} occurred at 12 hours post-dosing in males and 8 hours post-dosing in females, after which concentrations in both males and females decreased steadily up to 96 hours post-dosing.

Following a single oral administration of [^{14}C]chlorothalonil at 5 mg/kg bw, the major route of elimination was via the faeces, with 85–91% of the administered radioactivity recovered by 168 hours post-dosing. Urinary excretion accounted for 5.5–7.0% of the administered dose. Following a single oral administration of [^{14}C]chlorothalonil at 200 mg/kg bw, the major route of elimination was via the faeces, with 99–115% of the administered radioactivity recovered by 168 hours post-dosing. Urinary excretion accounted for less than 3.0% of the administered dose.

Following a single oral administration of [^{14}C]chlorothalonil at 5 mg/kg bw to bile duct–cannulated rats, the major route of elimination was via the faeces, with 75–80% of the administered radioactivity recovered by 72 hours post-dosing. Biliary elimination accounted for 12% of the administered dose, and urinary excretion accounted for 5.8–10% of the administered dose. Following a single oral administration of [^{14}C]chlorothalonil at 200 mg/kg bw to bile duct–cannulated rats, the major route of elimination was via the faeces, with 81–95% of the administered radioactivity recovered by 72 hours post-dosing. Biliary elimination accounted for 4.9–7.5% of the administered dose, and urinary excretion accounted for 2.9–4.3% of the administered dose (Punler, 2013).

In another study, [^{14}C]chlorothalonil (purity 98.6%) in 0.5% weight per volume (w/v) aqueous carboxymethylcellulose was dosed orally via gavage at 1.5 mg/kg bw or intravenously at 0.5 mg/kg bw

to groups of four male and four female Wistar rats per dose route. Excretion samples were obtained over a 7-day period. Blood samples were taken over a 3-day period to determine the pharmacokinetics of total radioactivity in blood.

Following a single oral administration of [^{14}C]chlorothalonil at 1.5 mg/kg bw, the majority of administered radioactivity (82–91%) was excreted in the first 24 hours, indicating that elimination was rapid. The [^{14}C]chlorothalonil that was systemically available, based on the urinary excretion ratio (oral:intravenous), accounted for 18–19% of the administered dose. Absorption was rapid, with peak mean measured blood concentrations observed at 2–4 hours. The oral bioavailability was approximately 2% for both sexes, suggesting that systemic availability was underestimated, as at least 5% of the dose was excreted in urine. No sex-related differences were observed in the pharmacokinetics or excretion of total radioactivity (Hutton, 2017).

1.2 Biotransformation

(a) In vivo

The nature and identity of the metabolites present in samples of plasma, urine, bile and faeces obtained from the study by Pulner (2013), described in section 1.1 above, were investigated.

Chlorothalonil was metabolized in the rat, forming up to seven metabolites through oxidation, hydroxylation and conjugation. For each matrix, metabolite profiles were qualitatively and quantitatively similar, regardless of sex and dose. The majority of the dose was excreted as unabsorbed parent in faeces. The major metabolites were identified as R611965 (3-carbamyl-2,4,5-trichlorobenzoic acid) and R613823 (2-acetamido-3-[3-(2-acetamido-3-hydroxy-3-oxo-propyl)sulfanyl-2,5-dichloro-4,6-dicyano-phenyl]sulfanyl-propanoic acid). Other identified components included SDS-3701 (R182281; 2,5,6-trichloro-4-hydroxyisophthalonitrile), R613825 (2-acetamido-3-[3,5-bis[(2-acetamido-3-hydroxy-3-oxo-propyl) sulfanyl]-4-chloro-2,6-dicyano-phenyl] sulfanyl-propanoic acid), R611966 (2,4,5-trichloro-3-cyanobenzamide), R417888 (2-carbamyl-3,5,6-trichloro-4-cyanobenzenesulfonic acid) and R419492 (4-carbamyl-2,5-dichloro-6-cyanobenzene-1,3-disulfonic acid). R613825 and R419492 had similar retention factors and could not be identified or quantified as individual metabolites.

The most abundant component excreted in urine was R613823 (≤3.3% of the dose). R611965 and the region comprising R613825 and/or R419492 accounted for 1.5% of the dose or less. A single component remained unidentified (≤1.0% of the dose), and origin-bound material accounted for 1.2% of the dose or less.

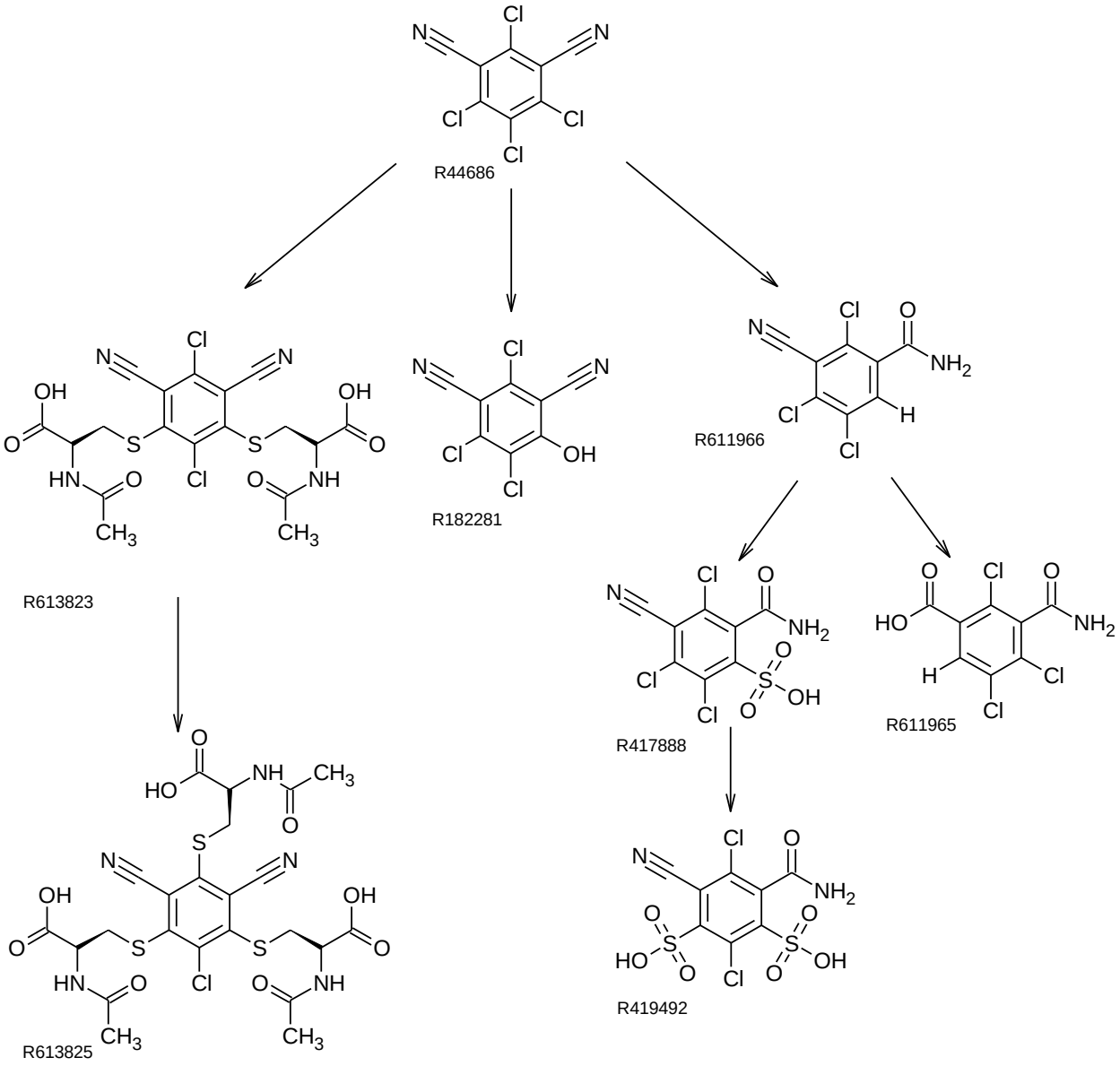
In bile, R613823 accounted for 1.7% of the dose or less, and R611965 and R613825/R419492 accounted for 1.4% of the dose or less. Three components in bile remained unidentified (≤2.0% of the dose), and origin-bound material accounted for 2.6% of the dose or less.

The most abundant component excreted in faeces was unabsorbed chlorothalonil (approximately 5.2–64.3% of the dose). R611966, SDS-3701, R611965, R613823 and R613825/R419492 were minor metabolites (≤5.4% of the dose). Two components remained unidentified (≤1.6% of the dose), and origin-bound material accounted for 4.4% of the dose or less.

The most abundant component circulating in plasma was SDS-3701, which accounted for 29–38% of the area under the concentration–time curve (AUC) for total radioactivity. The other circulating components observed were chlorothalonil, at 5.5–11% of the total radioactivity AUC, R611966, at 11–17% of the total radioactivity AUC, and R417888, at 4.7–12% of the total radioactivity AUC. Two components remained unidentified (4.1–13% of the total radioactivity AUC, ≤0.082 µg/g), and origin-bound material accounted for 7.8–16% of the total radioactivity AUC (≤0.181 µg/g) (Punler, 2013).

The proposed metabolic pathway for chlorothalonil in rats is shown in Fig. 2.

Fig. 2. Proposed metabolic pathway for chlorothalonil (R44686) in rats



Source: Punler (2013)

(b) In vitro

[^{14}C]Chlorothalonil (10 µmol/L) was incubated with rat and human liver microsomes at approximately 37 °C, and reactions were terminated following incubation for 0 and 60 minutes by the addition of acetonitrile acidified with formic acid (0.1% volume per volume). All samples were analysed by radio–high-performance liquid chromatography (HPLC), and fractions were labelled according to their approximate retention times. Each batch of microsomes was also incubated with [^{14}C]testosterone to assess the metabolic capacity of the test system used in the study.

The parent molecule accounted for more than 88% of the total chromatographic radioactivity after 60 minutes. The rank order for the disappearance of the [^{14}C]chlorothalonil parent molecule was female rat > male rat > human. Disappearance of the parent molecule was concomitant with the formation of up to six components across both species. All regions of radioactivity, except M3, M4 and M5, were considered to be of metabolic origin. M2 was the most notable component formed, accounting for 38.2% and 60.8% of the total chromatographic radioactivity following incubation for 60 minutes in

male and female rat liver microsomes, respectively. M2 was observed only in the 0-minute samples following incubation with human liver microsomes, and this component represented 3.93% of the total chromatographic radioactivity.

No unique or disproportionate human in vitro metabolites were considered to be formed under the experimental conditions employed (Sayer, 2017).

2. Toxicological studies

2.1 Short-term studies of toxicity

(a) Exposure by inhalation

In a 2-week inhalation toxicity study, a chlorothalonil formulation (Bravo WeatherStick 720 SC) containing 53.7% weight per weight (w/w) chlorothalonil was administered via nose-only inhalation (5 days per week, 6 hours per day) to six groups of 25 male Sprague Dawley rats at 0 mg/L (air control, group 1), 0 mg/L (blank formulation control, group 2), 0.001 mg/L (group 3), 0.003 mg/L (group 4), 0.009 mg/L (group 5) or 0.015 mg/L (group 6). Overall, the achieved chlorothalonil concentrations were 0.0011, 0.0029, 0.0096 and 0.0143 mg/L, respectively. The analytically measured mass median aerodynamic diameter was less than 4 μm.

Ten animals from each group were terminated after completion of 2 weeks of treatment, and five animals from each group were terminated at each of the designated recovery time points (48 hours, 7 days and 14 days after the last day of exposure). An additional group of five males (probe group 7) was exposed prior to all the other groups for a single 6-hour period to compare clinical signs with those observed in previous studies.

Animals were observed for clinical signs and mortality at selected time intervals. Body weights and feed consumption were measured at selected intervals. Ophthalmoscopy examinations were performed on all animals (except for probe group 7) pre-trial and on all control and high-dose animals during week 2 of treatment. Following termination after 2 weeks, all animals underwent a detailed necropsy examination, and the lungs were weighed. Respiratory tract tissues from all animals were retained and subjected to a comprehensive histopathological evaluation.

There were no premature decedents over the course of the study. There were some notable treatment-related clinical observations, including salivation (groups 2–6), staining of the muzzle (groups 5 and 6) and some respiratory signs (wheezing, gasping, irregular respiration; one or two animals in group 6 only). Concentration-dependent reduced body weight gains (Table 1) were found across all chlorothalonil treatment groups after the first and second weeks of exposure, with a net weight loss after the first week of exposure for group 6. During the recovery periods, the weight gain for the higher-concentration groups (groups 4–6) was slightly higher than for group 2.

Feed consumption followed a concentration-dependent trend, with statistically significant reductions for groups 4–6 during each of the two 5-day exposure periods. There were no ophthalmic findings considered to be related to treatment. No test item–related lung weight changes were noted. No treatment-related gross findings were noted. Microscopic findings in the larynx, lung, nasal cavity and trachea observed at all doses were indicative of irritation in the respiratory tract. At the end of the recovery period, the microscopic findings observed at the terminal euthanasia were not observed, were reduced in incidence and/or severity or showed partial recovery.

The no-observed-adverse-effect concentration (NOAEC) in the 2-week inhalation toxicity study was 0.001 mg/L, based on reduced body weight gain seen at 0.003 mg/L (Bain, 2013).

Table 1. Intergroup comparison of mean body weight change

Concentration (mg/L)	Mean body weight change (g)					
	Days 0–5	**Days 0–7**	**Days 0–12**	**Days 12–13**	**Days 12–19**	**Days 12–26**
0 (air control; group 1)	22	38	57	4	48	83
0 (blank formulation control; group 2)	18	35	54	5	45	79
0.001 (group 3)	17	34	50	5	47	84
0.003 (group 4)	10**	29*	43**	7	49	89
0.009 (group 5)	3**	25**	34**	8	56*	94
0.015 (group 6)	−3**	22**	25**	12**	60**	110

*: $P < 0.05$; **: $P < 0.01$
Source: Bain (2013)

2.2 Special studies

(a) Acute neurotoxicity

In a range-finding study of acute neurotoxicity, chlorothalonil (purity 99.3%) was administered orally by gavage as a single dose of 2000 mg/kg bw in 1.0% carboxymethylcellulose in water to male and female Crl:CD(SD) rats (four of each sex). A concurrent control group received the vehicle on a comparable dosing regimen. The dosing volume was 10 mL/kg bw for each group. All animals were observed twice daily for mortality and moribundity. Individual body weights and feed consumption were recorded daily beginning on the day of dose administration (study day 0). Detailed clinical observations were recorded for all animals at approximately 1, 2, 3, 4, 5, 6, 7 and 8 hours following dose administration on study day 0 and once daily thereafter until study day 6 (the day prior to scheduled euthanasia). All animals were euthanized and discarded without macroscopic examination on study day 7.

There were no treatment-related effects on clinical signs in rats of either sex. There was a statistically significant reduction in mean body weight in males on the first day of dosing compared with the control group. However, mean body weight gains for these animals were similar to those of the control group throughout the remainder of the study. Statistically significantly lower mean feed consumption was noted for 2000 mg/kg bw males and females following the first day of test substance administration, compared with the control group.

Based on the results of this study, the recommended high dose of chlorothalonil for evaluation in a subsequent acute neurotoxicity study was 2000 mg/kg bw, the limit dose for acute neurotoxicity testing (Herberth, 2014a).

In a study of acute neurotoxicity, chlorothalonil (purity 99.3%) was administered orally by gavage to groups of Crl:CD(SD) rats (10 of each sex per dose) at a dose of 100, 500 or 2000 mg/kg bw. A concurrent control group received the vehicle (1.0% carboxymethylcellulose in deionized water) on a comparable dosing regimen. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, except on days of functional observational battery (FOB) assessments. Individual body weights and feed consumption were recorded weekly beginning 1 week prior to dosing. FOB and locomotor activity data were recorded for all animals prior to dosing, at the time of peak effect (approximately 4–6 hours following dose administration) on study day 0, and on study days 7 and 14. On study day 15, five rats of each sex per group were deeply anaesthetized and perfused in situ; select tissues from the central and peripheral nervous systems were collected, and brain

weights and dimensions (excluding olfactory bulbs) were recorded. A neuropathological evaluation of nervous tissues collected from the five rats of each sex in the control and 2000 mg/kg bw groups was performed. The remaining (non-perfused) rats were euthanized and discarded without macroscopic examination.

There was no mortality. No test substance–related clinical findings were noted at any dose during the daily observations. Mean body weights, body weight gains and feed consumption were unaffected by test substance administration at all doses. No treatment-related effects on FOB or locomotor activity parameters were observed. No treatment-related effects on macroscopic or microscopic findings were observed.

The no-observed-adverse-effect level (NOAEL) for systemic toxicity and neurotoxicity was 2000 mg/kg bw, the highest dose tested (Herberth, 2014b).

(b) Short-term studies of neurotoxicity

In a 90-day neurotoxicity study, groups of 12 male and 12 female Alpk:APfSD (Wistar-derived) rats were fed diets containing chlorothalonil (purity 99.52%) at a concentration of 0, 30, 300 or 3000 parts per million (ppm) (equal to 0, 2.1, 22.0 and 232.1 mg/kg bw per day for males and 0, 2.4, 24.2 and 243.2 mg/kg bw per day for females, respectively). All animals were observed prior to the study start and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, were performed in weeks −1, 2, 5, 9 and 14. Locomotor activity was also monitored in weeks −1, 2, 5, 9 and 14. Body weights and feed consumption were recorded weekly throughout the study, and feed utilization was calculated over selected periods. At the end of the scheduled period, five rats of each sex per group were killed by in situ perfusion fixation, and selected nervous system tissues were removed, processed and examined microscopically.

No treatment-related mortality was observed. At 3000 ppm, there was a generalized yellow staining of the coat, limbs and ears and staining of the tray papers under the cages of these animals. Clinical signs of toxicity were confined to staining around the mouth and nose, which was also noted at necropsy. At the high dose, male body weights (adjusted for initial weight) were statistically significantly lower than those of controls (by up to 17% in weeks 10–14). The body weights of females fed 3000 ppm were 12% lower than those of controls in week 2 and remained statistically significantly lower than those of controls from week 5 onwards. There were no treatment-related effects on body weight in rats fed 30 or 300 ppm. At 3000 ppm, feed consumption was lower than that of the controls throughout the study in males and in week 1 and then from week 5 onwards in females. There was no effect of treatment on ophthalmoscopic examinations, FOB or motor activity parameters, brain weights, macroscopic findings or microscopic findings.

The NOAEL for systemic toxicity was 300 ppm (equal to 22.0 mg/kg bw per day), based on decreased body weights and reduced feed consumption seen at 3000 ppm (equal to 232.1 mg/kg bw per day). The NOAEL for neurotoxicity was 3000 ppm (equal to 232.1 mg/kg bw per day), the highest dose tested (Brammer, 2004, 2005).

(c) Immunotoxicity

In an immunotoxicity study, groups of 10 female CD-1 mice were fed diets containing chlorothalonil (purity 99%) at a concentration of 0, 50, 750 or 2000 ppm (equal to 0, 8.2, 120.9 and 345.1 mg/kg bw per day, respectively) for 28 days. An additional group of 10 females (positive controls) received cyclophosphamide, a known immunosuppressant, at 10 mg/kg bw per day by oral gavage for the same period. All animals received a single intravenous injection of the antigen, sheep red blood cells, with cell numbers of 2×10^8, on day 25. Animals were observed for adverse clinical signs throughout the study. Body weight and feed and water consumption were measured at several intervals. Blood was taken on day 29 for analysis of specific immunoglobulin M (IgM) to sheep red blood cells.

At terminal sacrifice, selected organs (liver, gall bladder, spleen, thymus and kidneys) were removed, weighed and preserved, but were not examined histopathologically.

There were no unscheduled deaths or clinical signs of toxicity. No treatment-related effects on body weight, feed consumption or water intake were observed. After administration of sheep red blood cells, there was no evidence that chlorothalonil had an immunosuppressant effect on the humoral system. The absolute kidney weights were statistically significantly increased at 750 and 2000 ppm. The kidney weights adjusted for body weight (covariate analysis) were 22% and 29% higher than the control values at 750 and 2000 ppm, respectively. There were no differences noted in liver, spleen or thymus weights. There were no abnormal findings noted at necropsy. Animals that received cyclophosphamide had a statistically significantly lower serum antibody-specific IgM, when compared with controls that received untreated diet alone.

The NOAEL for immunotoxicity was 2000 ppm (equal to 345.1 mg/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity was 50 ppm (equal to 8.2 mg/kg bw per day), based on increased kidney weights seen at 750 ppm (equal to 120.9 mg/kg bw per day) (Donald, 2012).

(d) Endocrine disruption

In an estrogen receptor binding assay, uterine cytosol from Sprague Dawley rats was used as the source of estrogen receptor to conduct binding experiments. A competitive binding experiment was conducted to measure the binding of a single concentration of [^{3}H]17β-estradiol (1 nmol/L) in the presence of increasing concentrations of chlorothalonil (purity 99.0%; logarithmic increase from 10^{-10} to 10^{-3} mol/L or 10^{-11} to 10^{-4} mol/L). Dimethyl sulfoxide (DMSO) was used as the vehicle at a final concentration of 2%. The assay included 19-norethindrone as a weak positive control, octyltriethoxysilane as a negative control and 17β-estradiol as the natural ligand reference material.

The reference compounds performed as expected in the assay. In the competitive binding experiment, chlorothalonil had no effect on specific binding at concentrations at or below 10^{-4} mol/L in runs 1 and 2 and at or below 10^{-5} mol/L in run 3. Precipitation occurred at 10^{-3} mol/L in runs 1 and 2 and at 10^{-4} mol/L in run 3.

Based on the results from the three runs, it was concluded that chlorothalonil is not active in the estrogen receptor binding assay (Willoughby, 2011a).

In an estrogen receptor transcriptional activation assay, hERa-HeLa-9903 cells cultured in vitro were exposed to chlorothalonil (purity 99.0%) at logarithmically increasing concentrations from 10^{-11} to 10^{-4} mol/L in cell culture medium containing 0.1% DMSO for 24 hours in two independent tests. The experiments were performed using 96-well plates, and each chlorothalonil concentration was tested in six replicates per plate. Cells were exposed to the test agent for 24 ± 2 hours to induce reporter (luciferase) gene products. Luciferase expression in response to activation of the estrogen receptor by chlorothalonil was measured using a proprietary luciferase assay.

The highest soluble concentration of chlorothalonil for use in the transcriptional activation assays was 10^{-4} mol/L, whereas the range of cytotoxicity was between 10^{-4} and 10^{-6} mol/L, as identified in the range-finding test and both independent runs. Because the maximum response relative to the positive control was less than 10% in at least two assay runs, chlorothalonil was considered to be negative for estrogen receptor transcriptional activation in this test system. The data indicate that chlorothalonil is not an estrogen receptor alpha agonist under the conditions of this study (Willoughby, 2011b).

In an androgen receptor binding assay, ventral prostate cytosol from Sprague Dawley rats was used as the source of androgen receptor to examine the ability of chlorothalonil to bind to the androgen receptor. The competitive binding assay was conducted to measure the binding of a single concentration of [^{3}H]R1881 (methyltrienolone; Chemical Abstracts Service No. 965-93-5; 10^{-9} mol/L; positive

control) in the presence of increasing concentrations (logarithmic increase from 10^{-11} to 10^{-3} mol/L) of chlorothalonil (purity 99.0%). DMSO was used as the solvent vehicle at a final assay concentration of approximately 3.2%. Three runs were performed in total, and each run included dexamethasone as a weak positive control and R1881 as the ligand reference standard.

The mean relative binding affinity (calculated by dividing the median inhibitory concentration [IC_{50}] of the positive control R1881 by the IC_{50} of the control/test material and multiplying by 100) was 0.0023% for the weak positive control (dexamethasone). As chlorothalonil was not classified as a "binder" (mean specific binding > 50%) for any of the runs, the relative binding affinity could not be calculated.

Based on the results from the three runs, it was concluded that chlorothalonil is a non-binder in the androgen receptor binding assay (Willoughby, 2012).

In a steroidogenesis assay, H295R cells cultured in vitro in 24-well plates were incubated with chlorothalonil (purity 99.0%) at logarithmic concentrations ranging from 10^{-10} to 10^{-4} mol/L for 48 ± 2 hours in triplicate for four independent experiments. DMSO was used as the solvent vehicle at a final concentration of 0.05%. A quality control plate containing two doses of the reference chemicals, forskolin (known inducer) and prochloraz (known inhibitor), was run each time the assay was performed. One independent experiment was not analysed because one of the forskolin concentrations on the quality control plate was different from the concentration specified in the test guideline (3.3 µmol/L was used instead of 1 µmol/L). Testosterone and estradiol levels were measured using HPLC with tandem mass spectrometry with internal standards.

Precipitation of the test compound was observed microscopically at 10^{-4} mol/L in runs 2 and 4. Cytotoxicity greater than 20% was observed at chlorothalonil concentrations of 10^{-5} and 10^{-4} mol/L in all three valid runs.

In three independent assays in H295R cells, chlorothalonil treatment did not result in statistically significant or reproducible alterations in testosterone or estradiol production (Wagner, 2012).

In an in vitro aromatase (cytochrome P450 19 [CYP19]) assay, chlorothalonil (purity 99%) in DMSO at logarithmic concentrations ranging from 10^{-10} to 10^{-3} mol/L was incubated with human recombinant aromatase and tritiated androstenedione ([1β-^{3}H]androst-4-ene-3,17-dione) for 15 minutes at 37 °C to assess the potential of chlorothalonil to inhibit aromatase activity. Aromatase activity was determined by measuring the amount of tritiated water produced at the end of a 15-minute incubation period for each concentration of chemical. Tritiated water was quantified using liquid scintillation counting.

Chlorothalonil was soluble in the assay buffer at concentrations of 10^{-4} mol/L and below. In three independent runs of the assay, increasing concentrations of chlorothalonil showed little decrease in aromatase activity. Chlorothalonil exhibited a mean aromatase activity of 89% (±1% standard deviation) at the highest soluble test concentration of 10^{-4} mol/L.

Under the study conditions utilized, chlorothalonil was not an inhibitor of aromatase activity (Wilga, 2011).

In a uterotrophic assay conducted to screen for potential estrogenic activity, chlorothalonil (purity 99.0%) in aqueous 1% carboxymethylcellulose was administered daily via oral gavage to groups of six ovariectomized Sprague Dawley rats at a dose of 0 (vehicle), 300 or 1000 mg/kg bw per day on postnatal days (PNDs) 65–67 or 66–68. A positive control group was treated with a daily dose of 17α-ethynyl estradiol in corn oil at 0.3 mg/kg bw per day by oral gavage. All animals were terminated and necropsied on PND 68 or 69 approximately 24 hours after administration of the last of the three doses to determine wet and blotted uterine weights.

Uterine weights in the chlorothalonil-treated groups at 300 and 1000 mg/kg bw per day were comparable to those of the vehicle controls. Absolute wet and blotted uterine weights for the positive control (17α-ethynyl estradiol) group were increased ($P \leq 0.01$) by 8.52- and 3.21-fold, respectively, as expected.

Based on the results of this study, it was concluded that chlorothalonil is negative in the uterotrophic assay (Sawhney Coder, 2012a).

In a Hershberger assay screening for androgenic activity, chlorothalonil (purity 99.0%) in aqueous 1% carboxymethylcellulose was administered daily via oral gavage to groups of six 54- to 56-day-old, castrated male Sprague Dawley rats at a dose of 300 or 1000 mg/kg bw per day (groups 4 and 5) for 10 days. Testosterone propionate (in corn oil), a known androgenic substance, was administered via subcutaneous injection at a dose of 0.2 mg/kg bw per day and a dosing volume of 0.5 mL/kg bw to group 2 males, which served as an androgenic comparator group for the test substance groups; the group 2 males also received a daily oral dose of the vehicle. Chlorothalonil and testosterone propionate were co-administered to groups 6, 7 and 8 at 100/0.2, 300/0.2 and 1000/0.2 mg/kg bw per day, respectively. The group 3 males received testosterone propionate comparably to the group 2 males, in conjunction with 3 mg/kg bw per day of flutamide (in corn oil), a known anti-androgenic substance, by daily oral gavage (at a dosing volume of 5 mL/kg bw), which served as an anti-androgenic comparator group for groups 6–8 to evaluate the potential of chlorothalonil to diminish the androgenic effects of testosterone propionate. A concurrent vehicle control group (group 1) received 0.5% carboxymethylcellulose orally by gavage.

All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and feed consumption were recorded daily. Blood was collected for possible future hormone analysis, and the bulbourethral glands, glans penis, liver, levator ani and bulbocavernosus muscle, seminal vesicles with coagulating glands and ventral prostate were examined, weighed and retained in 10% neutral-buffered formalin for possible future histopathological examination, with the following exception: the right portion of the medial lobe of the liver was flash-frozen and stored at approximately −70 °C for possible future biochemical analysis.

No test substance–related effects on mean body weight or body weight gain were noted in the 300 or 1000 mg/kg bw per day groups (groups 4 and 5). Co-administration of testosterone propionate at 0.2 mg/kg bw per day with chlorothalonil at 1000 mg/kg bw per day (group 8) resulted in slightly lower mean body weight gains sporadically throughout the treatment period (study days 0–10), compared with vehicle controls. As a result, the overall mean body weight gain in this group was lower (not statistically significantly) than in the testosterone propionate positive control group (group 2), and mean body weights were up to 6.22% lower than those in the testosterone propionate positive control group. Mean body weights and body weight gains were unaffected by co-administration of testosterone propionate at 0.2 mg/kg bw per day with chlorothalonil at 100 or 300 mg/kg bw per day (groups 6 and 7, respectively); differences from the testosterone propionate positive control group (group 2) were slight and not statistically significant. When the testosterone propionate positive control group (group 2) was compared with the vehicle control group (group 1), mean overall body weight gain in the testosterone propionate positive control group (78.0 g; study days 0–10) was statistically significantly higher than in the vehicle control group (65.5 g). No effects on mean body weight or body weight gain were noted when the testosterone propionate/flutamide positive control group (group 3) was compared with the testosterone propionate positive control group (group 2).

There was no effect of chlorothalonil treatment on the weight or histopathology of the bulbourethral glands, glans penis, liver, levator ani and bulbocavernosus muscle, seminal vesicles with coagulating glands or ventral prostate. No evidence of androgenic agonism or antagonism was noted following chlorothalonil administration at 300 or 1000 mg/kg bw per day alone (groups 4 and 5, respectively) or following co-administration of testosterone propionate at 0.2 mg/kg bw per day with chlorothalonil at 100, 300 or 1000 mg/kg bw per day (groups 6, 7 and 8, respectively).

Administration of the positive control substances, testosterone propionate and flutamide, gave the expected responses.

In conclusion, statistically significant changes were not seen in any of the five androgen-sensitive tissue weights (ventral prostate, seminal vesicle plus fluids and coagulating glands, levator ani and bulbocavernosus muscle, bulbourethral glands and glans penis). Chlorothalonil was negative for androgenicity and anti-androgenicity in the Hershberger assay (Sawhney Coder, 2012b).

(e) Phototoxicity

The phototoxic potential of chlorothalonil was determined in an in vitro cytotoxicity assay with the BALB/3T3 mouse fibroblast cell line. In this study, chlorothalonil (purity 98.6%) was dissolved in DMSO and diluted in a 1:100 ratio in Earle's balanced salt solution. The mouse fibroblast cells were treated for 1 hour at various concentrations of chlorothalonil solution at 37 ± 1 °C and for a further 50 minutes in the presence and absence of a non-toxic dose of ultraviolet A (UVA) light. One day after treatment, cytotoxicity was analysed as a measure of reduction of neutral red uptake and compared with the controls. DMSO was used as the negative control, and chlorpromazine (2-chloro-10-[3-dimethylaminopropyl] phenothiazine) was used as the positive control.

Chlorothalonil showed a cytotoxic effect with and without irradiation. The following median effective concentration (EC_{50}) values and photoirritation factor (PIF) were calculated:

−UVA: EC_{50} = 0.292 µg/mL

+UVA: EC_{50} = 0.149 µg/mL

PIF = 1.96

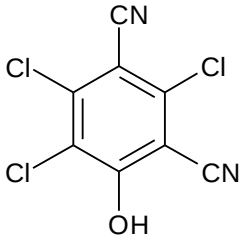
Based on the criterion in Organisation for Economic Co-operation and Development (OECD) Test Guideline 432 (i.e. PIF < 2), chlorothalonil is considered to produce no phototoxic effects (Gehrke, 2014).

3. Toxicological studies on metabolites and/or degradates

3.1 SDS-3701 (2,5,6-trichloro-4-hydroxyisophthalonitrile)

SDS-3701 (company codes R182281, DAC3701 and CNIL/02; Fig. 3) is found in soil (aerobic, anaerobic, photolysis), water (hydrolysis), crops (lettuce, tomato, carrot, wheat, rotated crops), livestock (hen, goat) and rats.

Fig. 3. Chemical structure of SDS-3701



(a) Acute toxicity

Lethal doses

In an acute oral toxicity study, SDS-3701 (purity 99.9%) was administered by oral gavage in propylene glycol as the vehicle to three female Wistar rats at 2000 mg/kg bw. Additional groups of three females were dosed at 300, 50 or 50 mg/kg bw. All animals were observed twice daily for clinical

signs and mortality for 2 weeks. Body weights were measured on days 1 (prior to administration), 8 and 15. All animals were subjected to macroscopic examination at termination.

At 2000 and 300 mg/kg bw, 3/3 and 2/3 animals died, while at 50 mg/kg bw, all animals survived. Clinical signs were observed at all doses and included hunched posture, uncoordinated movements, piloerection, lethargy and/or hyperthermia, as well as spasm and tremors at lethal doses. There were no effects of treatment on body weight. Macroscopic examination at termination revealed no test substance–related findings.

The oral median lethal dose (LD_{50} value) of SDS-3701 in female rats was identified to be between 50 and 300 mg/kg bw (Beerens-Heijnen, 2005a).

Dermal irritation

In a primary dermal irritation study, three adult male New Zealand white rabbits were each exposed via the dermal route to 0.5 g SDS-3701 (purity 99.9%). The test substance was moistened with 0.6 mL water and applied for 4 hours to the clipped skin of one flank using a semi-occlusive dressing. Observations were made 1, 24, 48 and 72 hours after exposure.

No dermal response was observed at the test site of any animal following exposure to SDS-3701, and no symptoms of systemic toxicity were found.

Based on the results of this study, it was concluded that SDS-3701 is not irritating to rabbit skin (Beerens-Heijnen, 2005b).

(b) Short-term studies of toxicity

Mice

In a repeated-dose toxicity study (performed pre-GLP), CD-1 mice (10 of each sex per dose) were administered SDS-3701 (purity 99.6%) in the diet at 0, 50, 125, 250, 500, 750 or 1500 ppm (equivalent to 0, 7.5, 18.75, 37.5, 75.0, 112.5 and 225.0 mg/kg bw per day, respectively) for 4 weeks. However, because of the lack of clearly defined effects after 2 weeks, doses of 3000 and 6000 ppm (equivalent to 450 and 900 mg/kg bw per day, respectively) were initiated at the beginning of the third week; this was accomplished by raising the doses of the 50 and 125 ppm groups, respectively. In order to evaluate the effects of the higher doses, the study was extended 1 additional week for a total duration of 5 weeks.

No treatment-related clinical signs of toxicity were observed. Four animals died spontaneously: one male at 50 ppm, one male and one female at 1500 ppm and one female at 3000 ppm. The deaths were not attributed to the treatment. In addition, three animals escaped from the cages. After dosing at 6000 ppm at week 3, animals of both sexes exhibited weight loss. Body weights were statistically significantly lower than control weights (>10%) at weeks 3 and 4 in females and at weeks 3, 4 and 5 in males. Body weights of the 3000 and 1500 ppm groups were reduced compared with those of controls (>10%) at some observation intervals in both sexes. Gross macroscopic examination did not reveal any treatment-related lesions (Ford & Killeen, 1980).

This study is not adequate to establish a NOAEL, as limited parameters were evaluated.

Rats

In a 13-week dietary toxicity study, SDS-3701 (purity 99.9%) was administered to Wistar Crl: (WI) BR (SPF) rats (10 of each sex per dose) at a concentration of 0, 15, 50 or 250 ppm (equal to 0, 1.0, 3.0 and 16 mg/kg bw per day for males and 0, 1.0, 4.0 and 17 mg/kg bw per day for females, respectively). The following parameters were evaluated: clinical signs daily; FOB and motor activity in week 12 or 13; body weight weekly; feed consumption twice weekly (weekly from week 6 onwards); ophthalmoscopy pretest and in week 12; and clinical pathology (haematology, clinical biochemistry and

urine analysis), macroscopy, selected organ weights and histopathology on a selection of tissues at termination.

Analytical measurements of the stability, homogeneity and accuracy of the target concentrations were made. Dietary analyses confirmed that diets were prepared accurately and homogeneously and were stable over at least 2 weeks at room temperature under normal laboratory light conditions.

No mortality occurred during the study. There were no treatment-related clinical signs of toxicity noted during the observation period. There were no treatment-related effects on FOB or motor activity parameters. No ophthalmological findings were noted in the treatment groups or controls.

Statistically significantly reduced body weights and body weight gains were observed in females at 250 ppm throughout the study starting from day 8 of the treatment. The body weights were reduced by about 12% in females compared with those of controls. The total difference in body weight gain over the treatment period compared with control body weight gain was approximately 35% in females at 250 ppm. The statistically significantly lower body weight gains of males at 50 and 250 ppm in the first week of treatment and of females at 15 ppm in week 12 were of a temporary and slight nature. Feed consumption was slightly reduced in females at 250 ppm throughout the treatment period. In females at 250 ppm, statistically significantly reduced red blood cell counts, haemoglobin and haematocrit levels and increased red cell distribution width were noted (Table 2). Red cell distribution width was also increased in males at 250 ppm, without concurrent changes in the other haematological parameters. Statistically significantly reduced total bilirubin levels were noted in males at 50 and 250 ppm and in females at 15 ppm and higher; the Meeting noted that decreased bilirubin is not considered to be an adverse effect in isolation. Increased creatinine levels were observed in males at 50 and 250 ppm, and reduced sodium levels were observed in males and females at 250 ppm (Table 3). Minor changes in these parameters at 50 ppm are not considered to be toxicologically relevant. No toxicologically relevant changes occurred in urine analysis parameters of treated rats. Macroscopic examination revealed no treatment-related abnormal findings. Slightly increased liver weights (absolute and relative to body weight) were observed in males at the high dose. In the absence of any changes in liver enzymes and histopathological findings, these increases in liver weights were considered adaptive and not adverse. Microscopic examination did not reveal any treatment-related lesions.

Table 2. Intergroup comparison of selected haematological parameters

	Males				**Females**			
Parameter	**0 ppm**	**15 ppm**	**50 ppm**	**250 ppm**	**0 ppm**	**15 ppm**	**50 ppm**	**250 ppm**
Red blood cells (10^{12}/L)	8.90	9.04	8.63	8.73	8.19	7.82	8.00	7.63*
Haemoglobin (mmol/L)	9.7	9.9	9.6	9.4	9.4	9.0*	9.2	8.3**
Haematocrit (L/L)	0.456	0.470	0.448	0.442	0.437	0.429	0.430	0.389**
RDW (%)	13.3	12.8	13.1	14.4**	12.3	13.5	12.4	14.6**

ppm: parts per million; RDW: red cell distribution width; *: $P < 0.05$; **: $P < 0.01$
Source: Van Otterdijk (2007a)

Table 3. Intergroup comparison of selected clinical chemistry parameters

	Males				Females			
Parameter	**0 ppm**	**15 ppm**	**50 ppm**	**250 ppm**	**0 ppm**	**15 ppm**	**50 ppm**	**250 ppm**
Total bilirubin (µmol/L)	2.5	2.2	1.9**	1.4**	3.3	2.2**	1.8**	1.5**
Creatinine (µmol/L)	39.1	41.7	44.9**	47.4**	47.2	49.7	51.1*	49.8
Sodium (mmol/L)	143.9	143.3	144.3	142.6*	142.6	141.8	142.4	140.8**

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$
Source: Van Otterdijk (2007a)

The NOAEL for SDS-3701 in this 90-day toxicity study in rats was 50 ppm (equal to 3.0 mg/kg bw per day), based on reduced body weight, body weight gain and feed consumption in females and changes in haematological and clinical chemistry parameters in both sexes at 250 ppm (equal to 16 mg/kg bw per day) (Van Otterdijk, 2007a).

In a 120-day dietary toxicity study (performed pre-GLP), SDS-3701 (purity assumed to be 100%) was administered to Sprague Dawley rats (10 males and 20 females per dose) at a concentration of 0, 10, 50, 100 or 200 ppm (equivalent to 0, 1, 5, 10 and 20 mg/kg bw per day, respectively). After 70 days, two female rats were housed with one male rat from the same dietary group for a 2-week period. These rats served as the parental generation in a (previously evaluated) three-generation reproductive toxicity study (Annex 1, reference *118*) conducted simultaneously with the 120-day feeding study.

Soft stool was observed in the 100 and 200 ppm groups. The 200 ppm group animals were observed to be nervous and irritable. The survival was 100% at all doses. The growth of males and females in the 10, 50 and 100 ppm groups and of males in the 200 ppm group was comparable to that of the control group. At the high dose, females displayed a decrease in growth rate from initiation up to mating. Body weight gain of the females at 200 ppm was statistically significantly decreased (>40%) in weeks 5–11 inclusive. Feed consumption was comparable in all dose groups. All clinical chemistry values were within the normal biological range, including a statistically significant elevation of alanine aminotransferase in high-dose animals. Haematological parameters were comparable in all groups. The results of urine analyses were comparable among all groups. Gross macroscopic findings were comparable among the groups. Liver weights (absolute and relative to body weight) were statistically significantly ($P < 0.05$) increased in the 200 ppm males compared with control values (adaptive and non-adverse response). No treatment-related histopathological findings were observed.

The NOAEL was 100 ppm (equivalent to 10 mg/kg bw per day), based on decreased body weight gains in females seen at 200 ppm (equivalent to 20 mg/kg bw per day) (Hastings & Jessup, 1975).

(c) Genotoxicity

The results of studies of the genotoxicity of SDS-3701 are summarized in Table 4. In vitro, SDS-3701 gave a positive response in only the mouse lymphoma assay, with and without metabolic activation. It was negative for genotoxicity in vivo in the mouse micronucleus test and for unscheduled DNA synthesis.

Table 4. Results of assays for the genotoxicity of SDS-3701

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 *Escherichia coli* WP2uvrA	3–5 000 µg/plate in DMSO (±S9)	99.9%	Negative	Verspeek-Rip (2004a)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	1–80 µg/mL in ethanol (−S9) 1–350 µg/mL in ethanol (+S9)	99.9%	Positive (±S9)[a]	Verspeek-Rip (2005a)
Mammalian cell gene mutation	Chinese hamster lung V79 cells; *Hprt* locus	3.9–31.3 µg/mL in DMSO (−S9) 7.8–125.0 µg/mL in DMSO (+S9)	98.7%	Negative	Chang (2017)
In vivo					
Micronucleus formation[b]	Male and female NMRI BR mice	500, 250 and 115 mg/kg bw (single oral gavage)	99.9%	Negative	Buskens (2004a)
Unscheduled DNA synthesis	Wistar Hanlbm:WIST (SPF)	250 and 500 mg/kg bw (gavage in corn oil)	99.9%	Negative	Honarvar (2005)

bw: body weight; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; *Hprt*: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

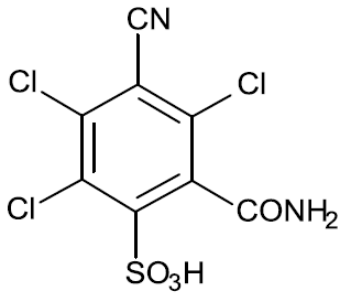
[a] Mutagenicity was observed only at highly cytotoxic concentrations.

[b] Proof of bone marrow exposure was demonstrated by Dunton (2016a).

3.2 R417888 (2-carbamyl-3,5,6-trichloro-4-cyanobenzenesulfonic acid)

R417888 (Fig. 4; company codes M12, VIS01, R6, Compound 10, U6 and CSCC890840) is found in soil (aerobic, anaerobic), crops (rotated crops) and rats.

Fig. 4. Chemical structure of R417888



(a) Acute toxicity

In an acute toxicity study, R417888 (purity 99.5%) was administered by gavage in water as vehicle to two groups of three female Wistar rats at 2000 mg/kg bw. All animals were observed twice daily for clinical signs and mortality for 2 weeks. Body weights were measured on days 1 (prior to administration), 8 and 15. All animals were subjected to macroscopic examination at termination.

No mortality occurred. Hunched posture was noted in all animals on days 1 and/or 2. There were no effects of treatment on body weight. No abnormalities were found at macroscopic examination of the animals.

In conclusion, the oral LD_{50} of R417888 in female Wistar rats was greater than 2000 mg/kg bw (Van Hygevoort, 2005).

(b) Short-term studies of toxicity

In a 90-day dietary toxicity study, R417888 (purity 100.0%) was administered to Wistar Crl: (WI) BR (SPF) rats (10 of each sex per dose) at a concentration of 0, 150, 600 or 2400 ppm (equal to 0, 13.0, 54.0 and 192 mg/kg bw per day for males and 0, 15.0, 56.0 and 218 mg/kg bw per day for females, respectively). The following parameters were evaluated: clinical signs daily; functional observational tests and motor activity in week 12; body weight weekly; feed consumption weekly (except weeks 4–9, when twice daily); ophthalmoscopy pretest (all animals) and in week 13 (controls and 2400 ppm); and clinical pathology (haematology, clinical biochemistry and urine analysis), macroscopy, selected organ weights and histopathology on a selection of tissues at termination.

Analytical measurements of stability, homogeneity and accuracy of the target concentrations were made. Dietary analyses confirmed that diets were prepared accurately and homogeneously and were stable over at least 2 weeks at room temperature under normal laboratory light conditions.

No mortality occurred during the study period. There were no clinical signs of toxicity noted during the observation period. Hearing ability, pupillary reflex, static righting reflex and grip strength were normal in all animals. The variation in motor activity did not indicate a relationship with treatment. There were no toxicologically relevant ophthalmology findings pre-dosing or in week 13. No toxicologically relevant changes in body weight or body weight gain were noted over the study period. Feed consumption before and after allowance for body weight was similar between treated and control animals. No toxicologically relevant changes occurred in haematological, clinical biochemistry or urine analysis parameters of treated rats. Necropsy did not reveal any toxicologically relevant alterations. No toxicologically relevant changes were noted in organ weights or organ to body weight ratios. There were no microscopic findings recorded that could be attributed to treatment with the test substance.

The NOAEL in the 90-day toxicity study in rats with R417888 was 2400 ppm (equal to 192 mg/kg bw per day), the highest dose tested (Van Otterdijk, 2007b).

(c) Genotoxicity

The results of genotoxicity tests with R417888 are shown in Table 5. R417888 gave a negative response for genotoxicity in several in vivo and in vitro assays, except for a positive response in the chromosomal aberration assay using human lymphocytes in the absence of metabolic activation and one mouse lymphoma assay with metabolic activation.

3.3 SYN548764 (4-carbamyl-2,3,5-trichloro-6-cyanobenzenesulfonic acid)

SYN548764 is a structural isomer of R417888 (see section 3.2 above). The sponsor noted that, historically, there had been some confusion between the identities of these two metabolites. The Ames, in vitro cytogenetics, mouse lymphoma and in vivo micronucleus study results originally conducted on what was thought to be SYN548764 were actually conducted with R417888. The new genotoxicity studies described below are reported to have been conducted with the correct metabolite (SYN548764). The actual chemical structure of SYN548764 is shown in Fig. 5.

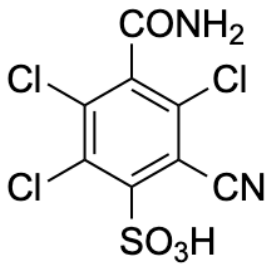
Table 5. Results of assays for the genotoxicity of R417888

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA	100–5 000 µg/plate in DMSO (±S9)	99.5%	Negative	Verspeek-Rip (2005b)
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA	3.0–5 000 µg/plate in DMSO (±S9)	95.0%	Negative	Sokolowski (2007)
Chromosomal aberrations	Human lymphocytes	14.3–2 200 µg/mL in DMSO (±S9)	95.0%	Positive (−S9) Negative (+S9)	Kunz (2007)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	333–3 000 µg/mL in ethanol (−S9) 3–1 000 µg/mL in ethanol (+S9)	99.5%	Positive (+S9)	Verspeek-Rip (2006)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	8.8–280.0 µg/mL in DMSO (−S9) 75–2 300 µg/mL in DMSO (+S9)	95.0%	Negative	Wollny (2007)
In vivo					
Micronucleus formation[a]	Five male NMRI BR mice	2000 mg/kg bw (single oral gavage in water)	99.5%	Negative	Meerts (2005)
Unscheduled DNA synthesis	Wistar Hanlbm:WIST (SPF) hepatocytes	1 000 and 2 000 mg/kg bw (gavage in PEG 400)	99.5%	Negative	Honarvar (2006)

bw: body weight; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; PEG: polyethylene glycol; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

[a] Proof of bone marrow exposure was demonstrated by Dunton (2016b).

Fig. 5. Chemical structure of SYN548764



SYN548764 (company code SYN548581) is found in soil (aerobic, anaerobic), crops (rotated crops) and rats.

(a) Genotoxicity

The results of genotoxicity assays with SYN548764 are shown in Table 6. SYN548764 gave a negative response for genotoxicity in in vivo and in vitro assays except for an equivocal response in the chromosomal aberration test in the absence of metabolic activation.

Table 6. Results of assays for the genotoxicity of SYN548764

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA pKM101 and WP2pKM101	3.0–5 000 µg/plate in DMSO (±S9)	99.5%	Negative	Sokolowski (2015a)
Chromosomal aberrations	Human lymphocytes	24.0–3 700 µg/mL in DMSO (±S9)	95.0%	Equivocal (−S9) Negative (+S9)	Sokolowski (2015b)[a]
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	200.0–1 400.0 µg/mL in deionized water (−S9) 231.3–3 700 µg/mL in deionized water (+S9)	95.0%	Negative	Wollny (2015a)
In vivo					
Micronucleus formation[b]	Male CD-1 mice	500, 1 000 and 2 000 mg/kg bw (single oral gavage in aqueous CMC with Tween 80)	95.0%	Negative	Dunton (2015a)

bw: body weight; CMC: 0.5% (w/v) aqueous carboxymethylcellulose; DMSO: dimethyl sulfoxide; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

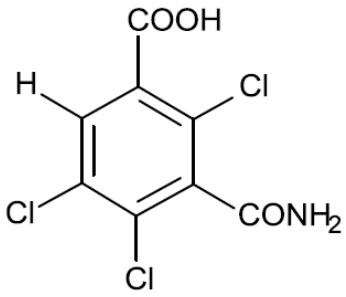
[a] Cytotoxicity at high dose.

[b] Proof of bone marrow exposure was demonstrated by Dunton (2015a).

3.4 R611965 (3-carbamyl-2,4,5-trichlorobenzoic acid)

R611965 (Fig. 6; company codes SDS-46851, M5, R14 and Compound 4) is found in soil (aerobic, anaerobic), crops (snap beans, rotated crops) and rats.

Fig. 6. Chemical structure of R611965



(a) Acute toxicity

Lethal doses

In an acute toxicity study, R611965 (purity 99.0%) was administered by oral gavage in 1% aqueous carboxymethylcellulose to two groups of three female Wistar Crl: (WI) BR (SPF) rats at 2000 mg/kg bw. All animals were observed twice daily for clinical signs and mortality for 2 weeks. Body weights were measured on days 1 (prior to administration), 8 and 15. All animals were subjected to macroscopic examination at termination.

No mortality occurred. Hunched posture was noted in all animals on days 1 and/or 2. There were no effects of treatment on body weight. No macroscopic abnormalities were observed.

In conclusion, the oral LD_{50} of R611965 in female Wistar rats was greater than 2000 mg/kg bw (Beerens-Heijnen, 2005c).

Dermal irritation

In a primary dermal irritation study, three adult male New Zealand white rabbits were each exposed via the dermal route to 0.5 g of R611965 (purity 99.0%). The test substance was moistened with water and applied for 4 hours to the clipped skin of one flank using a semi-occlusive dressing. Observations were made 1, 24, 48 and 72 hours after exposure.

No dermal response was observed at the test site of any animal following exposure to R611965, and no symptoms of systemic toxicity were observed.

Based on the results of this study, it was concluded that R611965 is not irritating to rabbit skin (Beerens-Heijnen, 2005d).

(b) Short-term studies of toxicity

In a 90-day toxicity study, R611965 (purity 99.1%) was administered to Wistar Crl: (WI) BR (SPF) rats (10 of each sex per dose) at a dietary concentration of 0, 150, 600 or 2400 ppm (equal to 0, 14.0, 57.0 and 197 mg/kg bw per day for males and 0, 14.0, 60.0 and 223 mg/kg bw per day for females, respectively). The following parameters were evaluated: clinical signs daily; FOB tests and motor activity in weeks 12–13; body weight weekly; feed consumption weekly (except weeks 3–9, when twice weekly); ophthalmoscopy pretest and in week 13; and clinical pathology (haematology, clinical biochemistry and urine analysis), macroscopy, selected organ weights and histopathology on a selection of tissues at termination.

Analytical measurements of stability, homogeneity and accuracy of the target concentrations were made. Dietary analyses confirmed that diets were prepared accurately and homogeneously and were stable over at least 2 weeks at room temperature under normal laboratory light conditions.

No toxicologically relevant changes were noted in any of the parameters investigated in this study at doses up to 2400 ppm.

The NOAEL was 2400 ppm (equal to 197 mg/kg bw per day), the highest dose tested (Van Otterdijk, 2007c).

(c) Genotoxicity

The results of genotoxicity assays with R611965 are shown in Table 7. R611965 gave a negative response for genotoxicity in all in vivo and in vitro assays conducted.

Table 7. Results of assays for the genotoxicity of R611965

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA	100.0–5 000 µg/plate in DMSO (±S9)	99.5%	Negative	Verspeek-Rip (2004b)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	1.0–2 660 µg/mL in DMSO (±S9)	99.0%	Negative	Verspeek-Rip (2005c)
In vivo					
Micronucleus formation	Male NMRI mice	2 000 mg/kg bw (single oral gavage in corn oil)	99.0%	Negative	Buskens (2004b)

bw: body weight; DMSO: dimethyl sulfoxide; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

(d) Relative potencies of chlorothalonil and R611965

In order to compare the potencies of chlorothalonil metabolite R611965 and chlorothalonil, the results of toxicological studies reviewed by this Meeting and by previous Meetings were compared (Table 8).

Table 8. Comparative toxicities of chlorothalonil and its metabolite R611965

	Chlorothalonil		**R611965**	
Study type	**NOAEL (mg/kg bw per day)**	**LOAEL (mg/kg bw per day)**	**NOAEL (mg/kg bw per day)**	**LOAEL (mg/kg bw per day)**
Acute oral toxicity (rat)	>5 000 mg/kg bw (LD_{50})	–	>5 000 mg/kg bw (LD_{50})	–
Ninety-day toxicity (mouse)	47.7	123.6	1 270	–
Thirty-day toxicity (rat)	–	80	2 000	–
Ninety-day toxicity (rat)	–	40	2 000	–
Ninety-day toxicity (rat)	10	40	197	–
Ninety-day toxicity (dog)	15	150	50	500
One-year toxicity (dog)	150	500	–	–
Two-year toxicity (dog)	3	–	–	–
Chronic toxicity (mouse)	–	40	1 022	–
Carcinogenicity (mouse)	–	40	1 022	–

Study type	Chlorothalonil		R611965	
	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Chronic toxicity (rat)	1.8	3.8	200	500
Carcinogenicity (rat)	2.7	10.6	1 000	–
Two-generation reproductive toxicity (rat)				
Parental toxicity	–	21.7	911	–
Reproductive toxicity	138	–	911	–
Offspring toxicity	68	138	911	–
Developmental toxicity (rat)				
Maternal toxicity	100	400	2 000	–
Embryo/fetal toxicity	100	400	2 000	–
Developmental toxicity (rabbit)				
Maternal toxicity	10	20	–	250
Embryo/fetal toxicity	20	–	500	1 000

bw: body weight; LD_{50}: median lethal dose; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level
Source: Current monograph; Annex 1, references *118* and *121*

The Meeting noted that in all studies except for the 90-day dog study, metabolite R611965 was at least 10 times less toxic than chlorothalonil. The three dog studies with chlorothalonil gave variable responses and showed that the dog was not the most sensitive species for chlorothalonil toxicity. The ratio between the lowest NOAEL for chlorothalonil (1.8 mg/kg bw per day) in the database and that for R611965 (50 mg/kg bw per day) was approximately 25.

3.5 R611968 (2,4,5-trichloro-3-cyano-6-hydroxybenzamide)

R611968 (Fig. 7; company codes Compound 5, SDS-47523 and R5) is found in soil (aerobic, anaerobic) and rats.

(a) Genotoxicity

The results of genotoxicity assays using R611968 are summarized in Table 8. R611968 was negative for genotoxicity in vitro in a bacterial gene mutation test and a mouse lymphoma assay, but gave a positive response in the chromosomal aberration assay in human lymphocytes, both with and without metabolic activation. It was negative for genotoxicity in an in vivo mouse micronucleus test.

Fig. 7. Chemical structure of R611968

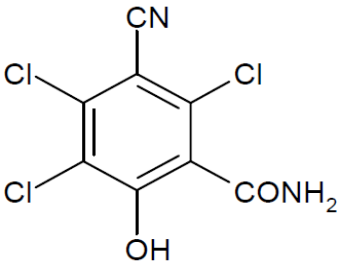


Table 8. Results of assays for the genotoxicity of R611968

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA pKM101 and WP2pKM101	3.0–5 000 µg/plate in DMSO (±S9)	99.5%	Negative	Sokolowski (2015c)
Chromosomal aberrations	Human lymphocytes	121.9–2 000 µg/mL in DMSO (−S9) 653.1–2 000 µg/mL in DMSO (+S9)	99.5%	Positive	Sokolowski (2015d)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	83.1–997.5 µg/mL in DMSO (−S9) 83.1–1 330 µg/mL in DMSO (+S9)	99.0%	Negative	Wollny (2015b)
In vivo					
Micronucleus formation[a]	Male CD-1 mice	125, 250 and 500 mg/kg bw (single oral gavage in CMC in Tween 80)	99.0%	Negative	Dunton (2015b)

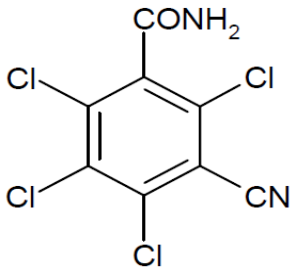
bw: body weight; CMC: 0.5% (w/v) aqueous carboxymethylcellulose; DMSO: dimethyl sulfoxide; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

[a] Proof of bone marrow exposure was demonstrated by Dunton (2015b).

3.6 R613636 (2,4,5,6-tetrachloro-3-cyanobenzamide)

R613636 (Fig. 8; company codes SDS-47525, M14, SDS-19221, R2, Compound 3 and CSCC548417) is a metabolite in soil, water and crops (rotated crops).

Fig. 8. Chemical structure of R613636



(a) Genotoxicity

The results of assays for the genotoxicity of R613636 are summarized in Table 9. R613636 was negative for genotoxicity in vitro in a bacterial gene mutation test, a gene mutation test in Chinese hamster lung cells and a mouse lymphoma assay, but gave a positive response in the chromosomal aberration test in human lymphocytes, both with and without metabolic activation. It was negative for genotoxicity in an in vivo mouse micronucleus test.

Table 9. Results of assays for the genotoxicity of R613636

End-point	Test object	Concentrations/doses	Purity	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2uvrA pKM101 and WP2pKM101	10.0–5 000 µg/plate in DMSO (±S9)	98.6%	Negative	Sokolowski (2015e)
Chromosomal aberrations	Human lymphocytes	13.2–40.3 µg/mL in DMSO (−S9) 13.2–59.6 µg/mL in DMSO (+S9)	98.6%	Positive	Sokolowski (2015f)
Mammalian cell gene mutation	*Tk* locus mouse lymphoma L5178Y	2.5–30.0 µg/mL in DMSO (−S9) 2.5–40.0 µg/mL in DMSO (+S9)	99.6%	Negative	Wollny (2015c)
Mammalian cell gene mutation	Chinese hamster lung V79 cells, *Hprt* locus	0.1–2.5 µg/mL in DMSO (−S9) 5.0–40.0 µg/mL in DMSO (+S9)	98.6%	Negative	Wollny (2015d)
In vivo					
Micronucleus formation[a]	Male CD-1 mice	312.5, 625 and 1 250 mg/kg bw (single oral gavage in CMC in Tween 80)	98.6%	Negative	Dunton (2015c)

bw: body weight; CMC: 0.5% (w/v) aqueous carboxymethylcellulose; DMSO: dimethyl sulfoxide; *Hprt*: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *Tk*: thymidine kinase

[a] Proof of bone marrow exposure was demonstrated by Dunton (2015c).

Comments

Biochemical aspects

Following a single oral gavage administration of [^{14}C]chlorothalonil at 5 mg/kg bw to rats, the major route of elimination was via the faeces, with 85–91% of the administered dose excreted by 168 hours post-dosing. Urinary excretion accounted for 5.5–7.0% of the administered dose. Following a single oral administration of [^{14}C]chlorothalonil at 200 mg/kg bw to rats, the major route of elimination was via the faeces, with 99–115% of the administered radioactivity recovered by 168 hours post-dosing. Urinary excretion accounted for less than 3.0% of the administered dose. Following a single oral administration of [^{14}C]chlorothalonil at 5 mg/kg bw to bile duct–cannulated rats, the major route of elimination was via the faeces, with 75–80% of the administered radioactivity recovered by 72 hours post-dosing. Biliary elimination accounted for 12% of the administered dose, and urinary excretion accounted for 5.8–10% of the administered dose. Following a single oral administration of [^{14}C]chlorothalonil at 200 mg/kg bw to bile duct–cannulated rats, the major route of elimination was via the faeces, with 81–95% of the administered radioactivity recovered by 72 hours post-dosing. Biliary elimination accounted for 4.9–7.5% and urinary excretion accounted for 2.9–4.3% of the administered dose (Punler, 2013).

Following administration of a single oral gavage dose of [^{14}C]chlorothalonil at 5 mg/kg bw to rats, the C_{max} of radioactivity in blood was 0.21 µg equiv/g in males and 0.31 µg equiv/g in females, with a T_{max} of 8 hours post-dosing in males and 4 hours post-dosing in females. Following administration of a single oral gavage dose of 200 mg/kg bw, the C_{max} values in blood were 3.2 and 6.0 µg equiv/g in males and females, respectively, with a T_{max} of 12 hours post-dosing in both males and females (Punler, 2013).

Chlorothalonil was metabolized in the rat, forming up to seven metabolites through oxidation, hydroxylation and conjugation. The majority of the dose was excreted as unabsorbed parent in faeces. The most abundant component excreted in urine was R613823 (≤3.3% of the dose). The most abundant metabolite in plasma (29–38% of the total radioactivity AUC) was SDS-3701 (Punler, 2013). In an in vitro metabolism study using rat and human liver microsomes, no qualitative differences in the metabolite profiles of [^{14}C]chlorothalonil were noted, and no unique or disproportionate human in vitro metabolites were formed (Sayer, 2017).

Toxicological data

In a study of acute neurotoxicity, chlorothalonil was administered by gavage to groups of rats at a dose of 0, 100, 500 or 2000 mg/kg bw. The NOAEL for systemic toxicity and neurotoxicity was 2000 mg/kg bw, the highest dose tested (Herberth, 2014b).

In a 90-day neurotoxicity study, groups of rats were fed diets containing chlorothalonil at a concentration of 0, 30, 300 or 3000 ppm (equal to 0, 2.1, 22.0 and 232.1 mg/kg bw per day for males and 0, 2.4, 24.2 and 243.2 mg/kg bw per day for females, respectively). The NOAEL for systemic toxicity was 300 ppm (equal to 22.0 mg/kg bw per day), based on decreased body weights and reduced feed consumption at 3000 ppm (equal to 232.1 mg/kg bw per day). The NOAEL for neurotoxicity was 3000 ppm (equal to 232.1 mg/kg bw per day), the highest dose tested (Brammer, 2004).

The Meeting concluded that chlorothalonil is not neurotoxic.

In an immunotoxicity study, groups of female mice were fed diets containing chlorothalonil at a concentration of 0, 50, 750 or 2000 ppm (equal to 0, 8.2, 120.9 and 345.1 mg/kg bw per day, respectively) for a period of 28 days. The NOAEL for immunotoxicity was 2000 ppm (equal to 345.1 mg/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity was 50 ppm (equal to 8.2 mg/kg bw per day), based on increased kidney weights at 750 ppm (equal to 120.9 mg/kg bw per day) (Donald, 2012).

The Meeting concluded that chlorothalonil is not immunotoxic.

No evidence of chlorothalonil-mediated estrogenic or androgenic activity was observed in a battery of in vivo and in vitro tests (Wilga, 2011; Willoughby, 2011a,b, 2012; Wagner, 2012; Sawhney Coder, 2012a,b).

Chlorothalonil is considered to be non-phototoxic in vitro (Gehrke, 2014).

Toxicological data on metabolites and/or degradates

SDS-3701 (2,5,6-trichloro-4-hydroxyisophthalonitrile; plant, ruminant and rat metabolite and soil and water degradate)

The oral LD_{50} of SDS-3701 in rats was between 50 and 300 mg/kg bw (Beerens-Heijnen, 2005a). SDS-3701 was not irritating to rabbit skin (Beerens-Heijnen, 2005b).

In a 13-week dietary toxicity study, SDS-3701 was administered to rats at a concentration of 0, 15, 50 or 250 ppm (equal to 0, 1.0, 3.0 and 16 mg/kg bw per day for males and 0, 1.0, 4.0 and 17 mg/kg bw per day for females, respectively). The NOAEL was 50 ppm (equal to 3.0 mg/kg bw per day), based on reduced body weights, body weight gains and feed consumption in females and changes in haematological and clinical chemistry parameters seen in both sexes at 250 ppm (equal to 16 mg/kg bw per day) (Van Otterdijk, 2007a).

In a 120-day dietary toxicity study (pre-GLP), SDS-3701 was administered to rats at a concentration of 0, 10, 50, 100 or 200 ppm (equivalent to 0, 1, 5, 10 and 20 mg/kg bw per day, respectively). The NOAEL was 100 ppm (equivalent to 10 mg/kg bw per day), based on decreased body weight gains in females at 200 ppm (equivalent to 20 mg/kg bw per day) (Hastings & Jessup, 1975).

SDS-3701 was tested for genotoxicity in vitro in a bacterial gene mutation test (Verspeek-Rip, 2004a), a forward mutation test in Chinese hamster lung cells (Chang, 2017) and a mouse lymphoma assay (Verspeek-Rip, 2005a), with and without metabolic activation. It gave a positive response in the in vitro mouse lymphoma assay only, with and without metabolic activation. It was negative for genotoxicity in vivo in the mouse micronucleus test (Buskens, 2004a) and for unscheduled DNA synthesis (Honarvar, 2005). The Meeting confirmed the previous conclusion of the 2009 Meeting that SDS-3701 is unlikely to exhibit mutagenic activity in vivo.

R417888 (2-carbamyl-3,5,6-trichloro-4-cyanobenzenesulfonic acid; crop and rat metabolite and soil degradate)

The acute oral LD_{50} of R417888 in rats was greater than 2000 mg/kg bw (Van Hygevoort, 2005).

In a 90-day dietary toxicity study, R417888 was administered to rats at a concentration of 0, 150, 600 or 2400 ppm (equal to 0, 13.0, 54.0 and 192 mg/kg bw per day for males and 0, 15.0, 56.0 and 218 mg/kg bw per day for females, respectively). The NOAEL was 2400 ppm (equal to 192 mg/kg bw per day), the highest dose tested (Van Otterdijk, 2007b).

R417888 was tested for genotoxicity in vitro in bacterial gene mutation tests (Verspeek-Rip, 2005b; Sokolowski, 2007), a chromosomal aberration test in human lymphocytes (Kunz, 2007) and mouse lymphoma assays (Verspeek-Rip, 2006; Wollny, 2007), with and without metabolic activation. It gave a positive response in the chromosomal aberration assay without metabolic activation (Kunz, 2007) and one mouse lymphoma assay with metabolic activation (Verspeek-Rip, 2006), but it was negative in a repeat mouse lymphoma assay at higher concentrations (Wollny, 2007). It was negative for genotoxicity in vivo in a mouse micronucleus test (Meerts, 2005) and for unscheduled DNA synthesis (Honarvar, 2006).

The Meeting concluded that R417888 is unlikely to be genotoxic in vivo.

SYN548764 (4-carbamyl-2,3,5-trichloro-6-cyanobenzenesulfonic acid; crop and rat metabolite and soil degradate)

SYN548764 was tested for genotoxicity in vitro in a bacterial gene mutation test (Sokolowski, 2015a), a mouse lymphoma assay (Wollny, 2015a) and a chromosomal aberration test in human lymphocytes (Sokolowski, 2015b), with and without metabolic activation. It gave an equivocal response in the chromosomal aberration test only, in the absence of metabolic activation. It was negative for genotoxicity in the in vivo mouse micronucleus test (Dunton, 2015a).

The Meeting concluded that SYN548764 is unlikely to be genotoxic in vivo.

R611965 (3-carbamyl-2,4,5-trichlorobenzoic acid; crop and rat metabolite and soil degradate)

The acute oral LD_{50} of R611965 in rats was greater than 2000 mg/kg bw (Beerens-Heijnen, 2005c). R611965 was not irritating to rabbit skin (Beerens-Heijnen, 2005d).

In a 90-day toxicity study, R611965 was administered to rats at a dietary concentration of 0, 150, 600 or 2400 ppm (equal to 0, 14.0, 57.0 and 197 mg/kg bw per day for males and 0, 14.0, 60.0 and 223 mg/kg bw per day for females, respectively). The NOAEL was 2400 ppm (equal to 197 mg/kg bw per day), the highest dose tested (Van Otterdijk, 2007c).

R611965 was negative in vitro in a bacterial gene mutation test (Verspeek-Rip, 2004b) and a mouse lymphoma assay (Verspeek-Rip, 2005c), with and without metabolic activation. It was negative for genotoxicity in the in vivo mouse micronucleus test (Buskens, 2004b). The Meeting confirmed the conclusion of the 2010 Meeting that R611965 is unlikely to be genotoxic.

R611968 (2,4,5-trichloro-3-cyano-6-hydroxybenzamide; rat metabolite and soil degradate)

R611968 was tested for genotoxicity in vitro in a bacterial gene mutation test (Sokolowski, 2015c), a chromosomal aberration test in human lymphocytes (Sokolowski, 2015d) and a mouse lymphoma assay (Wollny, 2015b), with and without metabolic activation. It gave a positive response in the chromosomal aberration assay (Sokolowski, 2015d) only, both with and without metabolic activation. It was negative for genotoxicity in an in vivo mouse micronucleus test (Dunton, 2015b).

The Meeting concluded that R611968 is unlikely to be genotoxic in vivo.

R613636 (2,4,5,6-tetrachloro-3-cyanobenzamide; crop metabolite and soil and water degradate)

R613636 was tested for genotoxicity in vitro in a bacterial gene mutation test (Sokolowski, 2015e), a gene mutation test in Chinese hamster lung cells (Wollny, 2015d), a chromosomal aberration test in human lymphocytes (Sokolowski, 2015f) and a mouse lymphoma assay (Wollny, 2015c), with and without metabolic activation. It gave a positive response in the chromosomal aberration test only, both with and without metabolic activation. It was negative for genotoxicity in an in vivo mouse micronucleus test (Dunton, 2015c).

The Meeting concluded that R613636 is unlikely to be genotoxic in vivo.

Toxicological evaluation

The Meeting concluded that no revision of the ADI or ARfD for chlorothalonil was necessary.

The Meeting concluded that no revision of the ADI or ARfD for metabolite SDS-3701 was necessary.

For metabolite R611965, the Meeting concluded that it would be covered by the ADI and ARfD of chlorothalonil, but noted that it is at least 10 times less potent than chlorothalonil in a wide range of studies.

The Meeting concluded that metabolite R417888 would be covered by the ADI and ARfD of chlorothalonil because of its lower acute and repeated-dose toxicity in comparison with the parent compound.

The Meeting concluded that SYN548764, R611968 and R613636 are unlikely to be genotoxic in vivo. Following the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015), the Meeting concluded that, for chronic toxicity, these three metabolites could be assessed using the threshold of toxicological concern (TTC) approach. All three metabolites are categorized in Cramer class III, and therefore a TTC of 1.5 µg/kg bw per day applies.

The ADI for chlorothalonil is 0–0.02 mg/kg bw. This ADI applies to chlorothalonil plus the metabolites R611965 and R417888, expressed as chlorothalonil.

The ARfD for chlorothalonil is 0.6 mg/kg bw. This ARfD applies to chlorothalonil plus the metabolites R611965 and R417888, expressed as chlorothalonil.

Acceptable daily intake (ADI) (applies to chlorothalonil plus R611965 and R417888, expressed as chlorothalonil)

0–0.02 mg/kg bw

Acute reference dose (ARfD) (applies to chlorothalonil plus R611965 and R417888, expressed as chlorothalonil)

0.6 mg/kg bw

Critical end-points for setting guidance values for exposure to chlorothalonil and metabolites

Short-term studies of toxicity	
Target/critical effect	Body weight
Lowest relevant inhalation NOAEC	0.001 mg/L (rat)
Neurotoxicity	
Acute neurotoxicity NOAEL	2 000 mg/kg bw, highest dose tested (rat)
Subchronic neurotoxicity NOAEL	232.1 mg/kg bw per day, highest dose tested (rat)
Other toxicological studies	
Immunotoxicity NOAEL	345 mg/kg bw per day, highest dose tested (mouse)
Endocrine effects	No evidence in in vitro or in vivo tests
Phototoxicity	Not phototoxic in vitro
Studies on metabolites	
SDS-3701	
Acute toxicity	
Rat, LD_{50}, oral	$50 < LD_{50} < 300$ mg/kg bw
Rabbit, dermal irritation	Not irritating
Short-term studies of toxicity	
Target/critical effect	Body weight, body weight gain, changes in haematology and clinical chemistry parameters
Lowest relevant oral NOAEL	3 mg/kg bw per day (rat)
Genotoxicity	

	No evidence of genotoxicity in vivo
R417888	
Acute toxicity	
Rat, LD_{50}, oral	>2 000 mg/kg bw (rat)
Short-term studies of toxicity	
Target/critical effect	None
Lowest relevant oral NOAEL	192 mg/kg bw per day, highest dose tested (rat)
Genotoxicity	
	No evidence of genotoxicity in vivo
SYN548764	
Genotoxicity	
	No evidence of genotoxicity in vivo
R611965	
Acute toxicity	
Rat, LD_{50}, oral	>2 000 mg/kg bw
Rabbit, dermal irritation	Not irritating
Short-term studies of toxicity	
Target/critical effect	None
Lowest relevant oral NOAEL	197 mg/kg bw per day, highest dose tested (rat)
Genotoxicity	
	No evidence of genotoxicity
R611968	
Genotoxicity	
	No evidence of genotoxicity in vivo
R613636	
Genotoxicity	
	No evidence of genotoxicity in vivo

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CYPRODINIL (addendum)

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Explanation

Cyprodinil (4-cyclopropyl-6-methyl-*N*-phenylpyrimidin-2-amine) was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2003, when an acceptable daily intake (ADI) of 0–0.03 mg/kg body weight (bw) was established. The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for cyprodinil (Annex 1, reference *100*).

The 2003 Meeting also assessed data on four metabolites of cyprodinil: CGA275535, CGA249287, CGA321915 and NOA422054. The 2003 Meeting concluded that the acute toxicity of these four metabolites was low, and no mutagenic potential was detected (Annex 1, reference *100*).

Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, cyprodinil was placed on the agenda of the present Meeting, which assessed additional toxicological information available since the last review. In addition, the Meeting applied JMPR's "Plant and animal metabolite assessment scheme" (WHO, 2015) for the assessment of the metabolites of cyprodinil.

A number of new toxicological studies on cyprodinil were submitted to the present Meeting, including studies on metabolism, phototoxicity, dermal toxicity, genotoxicity and immunotoxicity, as well as genotoxicity studies on metabolites.

All critical studies contained statements of compliance with good laboratory practice and were conducted in accordance with relevant national or international test guidelines, unless otherwise specified. No additional information from a literature search was identified that complemented the toxicological information submitted for the current assessment.

Evaluation for acceptable intake

1. Biochemical aspects

1.1 Biotransformation

(a) In vitro comparative metabolism in human and rat liver microsomes

In vitro metabolic profiling of cyprodinil was carried out by incubating human and rat liver microsomes (0.5 mg protein/mL) with [phenyl-U-^{14}C]cyprodinil (purity 99%) at a concentration of 10 µmol/L and a reduced nicotinamide adenine dinucleotide phosphate (NADPH) regenerating system for 60 minutes at 37 °C. The study was conducted with a mixed-sex pool of human liver microsomes and separated pools of male and female Wistar rat liver microsomes. Time zero and negative controls without microsomes and without the NADPH regenerating system were also used in order to determine the stability of cyprodinil in the incubation medium and to validate the NADPH-dependent metabolism. Positive controls (i.e. testosterone 6β-hydroxylation and 7-ethoxycoumarin *O*-dealkylation enzymatic activities) were used to check the metabolic competences of the human and rat liver microsomes, respectively.

An extensive NADPH-dependent metabolism of cyprodinil was observed in human and rat liver microsomes after 60 minutes of incubation. Remaining parent compound accounted for 31.6% of the dose in humans and for 5.5% and 6.8% of the dose in male and female rats, respectively. Up to 14 radio–high-performance liquid chromatography (HPLC) peaks (P1–P14) were observed in human and rat liver microsome incubates. P12 was assigned to unchanged cyprodinil. P1–P11 were produced by NADPH-dependent metabolism of cyprodinil in both humans and rats. P7 was the main metabolite in humans and rats. In humans, P7 accounted for 61% of the dose after 60 minutes of incubation; the other metabolic peaks accounted for less than 5% of the dose. In rats, P7 accounted for 59.6% (males) or 71.7% (females) of the dose. In male rats, two other metabolites accounted for more than 5% of the dose: P4 (12.4%) and P6 (5.9%). In female rats, P4 accounted for 8.7% of the dose. These results are summarized in Table 1.

Positive and negative controls gave the appropriate responses.

In conclusion, cyprodinil was extensively metabolized in human and rat liver microsomes in vitro. No human-specific metabolites were detected (Thibaut, 2017).

Table 1. Comparison of metabolic profiles of [^{14}C]cyprodinil in human and rat liver microsomes after 60 minutes of incubation

	% of ROIs[a]		
	Humans	**Male rats**	**Female rats**
HPLC peak	**Mean ± SD *(RRT)***	**Mean ± SD *(RRT)***	**Mean ± SD *(RRT)***
P1	0.8 ± 0.0 *(0.07)*	1.9 ± 0.1 *(0.07)*	1.3 ± 0.2 *(0.07)*
P2	nd	2.4 ± 0.2 *(0.46)*	nd
P3	0.3 ± 0.4 *(0.60)*	4.3 ± 0.3 *(0.60)*	2.4 ± 0.3 *(0.60)*
P4	1.7 ± 0.1 *(0.62)*	12.4 ± 0.2 *(0.62)*	8.7 ± 0.3 *(0.62)*
P5	nd	0.2 ± 0.4 *(0.67)*	nd
P6	nd	5.9 ± 0.9 *(0.69)*	3.5 ± 0.2 *(0.69)*
P7	61.0 ± 0.4 *(0.80)*	59.6 ± 0.7 *(0.80)*	71.7 ± 0.7 *(0.80)*
P8	1.2 ± 0.2 *(0.83)*	1.6 ± 0.4 *(0.83)*	1.5 ± 0.2 *(0.83)*
P9	1.3 ± 0.2 *(0.86)*	2.6 ± 0.2 *(0.86)*	1.1 ± 0.2 *(0.86)*
P10	2.2 ± 0.2 *(0.88)*	1.9 ± 0.1 *(0.88)*	3.2 ± 0.3 *(0.88)*

	% of ROIs[a]		
	Humans	**Male rats**	**Female rats**
HPLC peak	**Mean ± SD *(RRT)***	**Mean ± SD *(RRT)***	**Mean ± SD *(RRT)***
P11	nd	1.7 ± 0.2 *(0.91)*	nd
P12 (parent)	30.8 ± 0.3 *(1.00)*	5.4 ± 0.1 *(1.00)*	6.7 ± 0.4 *(1.00)*
P13	0.8 ± 0.1 *(1.02)*	nd	nd
P14	nd	nd	nd

HPLC: high-performance liquid chromatography; nd: not detected; ROIs: regions of interest; RRT: relative retention time (mean value); SD: standard deviation

[a] Results are expressed as mean ± SD of four replicates.

Source: Thibaut (2017)

2. Toxicological studies

2.1 Short-term studies of toxicity

(a) Dermal application

Groups of 10 male and 10 female HanRcc: WIST (SPF) rats were dermally administered cyprodinil (purity 99.2%; batch/lot no. P.012011) in 0.5% weight per volume (w/v) aqueous carboxymethylcellulose in 0.1% w/v polysorbate 80 under semi-occlusive conditions at a dose of 0, 5, 25, 125 or 1000 mg/kg bw per day, 6 hours per day, 5 days per week, over a period of 28 days.

There were no treatment-related findings on mortality, treated skin site, general or detailed clinical observations, ophthalmological examination, functional observational battery tests or grip strength measurements. One female at 25 mg/kg bw per day was euthanized following an accident. Locomotor activity measurements showed a slight increase in motor activity in males and females treated at 1000 mg/kg bw per day during the first 10 minutes of the test run only. There were no treatment-related effects on feed consumption, body weight, haematology, clinical biochemistry or histopathology of the treated skin or selected organ and tissue samples in either sex. The slight increase in locomotor activity was not considered to be adverse owing to its transient nature and lack of a similar effect following oral exposure to cyprodinil at higher doses in previously evaluated acute and 90-day neurotoxicity studies in rats (Annex 1, reference *100*).

The no-observed-adverse-effect level (NOAEL) for dermal toxicity in rats administered cyprodinil under semi-occlusive conditions was 1000 mg/kg bw per day, the highest dose tested (Sommer, 2008).

2.2 Genotoxicity

The results of in vitro genotoxicity studies with cyprodinil are summarized in Table 2.

The reverse mutation assay was performed to investigate the potential of cypronidil (purity 99.9%; lot/batch no. SMU9AP001) to induce gene mutations in the plate incorporation test (experiment I) and the preincubation test (experiment II) using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strains WP2 uvrA pKM 101 and WP2 pKM 101 over the following ranges: experiments I and II: 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate; experiment IIA: 1, 3, 10, 33, 100 and 333 µg/plate; and experiment IIB: 0.1, 0.3, 1, 3, 10, 33, 100 and 333 µg/plate. Reduced background growth and distinct toxic effects were observed at the higher concentrations with and without metabolic activation in nearly all strains used in all four experiments.

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with cyprodinil at any dose in the presence or absence of metabolic activation (S9). The positive control chemicals induced appropriate responses in the corresponding strains.

Table 2. Summary of genotoxicity tests with cyprodinil

End-point	Test object	Concentrations/doses	Results	Purity / lot/batch no.	Reference
In vitro					
Reverse mutation assay	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) *Escherichia coli* (WP2 uvrA pKM 101 and WP2 pKM 101)	*With/without S9* Exp. I and II: 3, 10, 33, 100, 333, 1 000, 2 500, 5 000 µg/plate Exp. IIA: 1, 3, 10, 33, 100, 333 µg/plate Exp. IIB: 0.1, 0.3, 1, 3, 10, 33, 100, 333 µg/plate	Negative	99.9% / SMU9AP001	Sokolowski (2009)
Gene mutation	Chinese hamster lung (V79) cells (*Hprt* locus)	*Without S9* 2.0, 4.0, 8.0, 16.0, 24.0 µg/mL *With S9* 8.0, 16.0, 32.0, 48.0, 64.0 µg/mL	Negative	99.2% / P.012011	Wollny (2017)
Micronucleus test	Human lymphocytes	*Without S9* Exp. IA: 7.7, 13.5, 23.7, 41.5 µg/mL (4 hours) Exp. IC: 45.7, 48.0, 50.4, 52.9 µg/mL (4 hours) Exp. IIB: 22.8, 45.7, 47.9, 50.3 µg/mL (20 hours) *With S9* Exp. IA: 13.5, 23.7, 41.5, 72.6 µg/mL (4 hours) Exp. IB: 40.4, 52.5, 68.3, 88.8 µg/mL (4 hours)	Negative	99.2% / P.012011	Naumann (2017a)

Exp.: Experiment; *Hprt*: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate

Under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, cyprodinil is considered to be non-mutagenic in this *S. typhimurium* and *E. coli* reverse mutation assay (Sokolowski, 2009).

The potential for cyprodinil (purity 99.2%; lot/batch no. P.012011) to induce gene mutations at the *Hprt* locus using V79 cells of the Chinese hamster was investigated in an in vitro study. The assay was performed in one experiment using two parallel cultures, a treatment period of 4 hours and the presence or absence of liver microsomal activation. The maximum cyprodinil concentration in the pre-experiment was 2000 µg/mL. The concentration range of the main experiment was limited by precipitation and cytotoxicity of the test item. The main experiment was evaluated at concentrations of 2.0, 4.0, 8.0, 16.0 and 24.0 µg/mL without metabolic activation and 8.0, 16.0, 32.0, 48.0 and 64.0 µg/mL with metabolic activation.

Cytotoxic effects occurred at 24.0 µg/mL without metabolic activation and at 64.0 µg/mL with metabolic activation. No relevant or reproducible increase in mutant colony numbers per 10^6 cells was observed in the main experiment up to the maximum concentration. The mutation frequency (mean

values of both parallel cultures) remained within the 95% control limit with and without metabolic activation.

Ethyl methanesulfonate and 7,12-dimethylbenz[*a*]anthracene, used as positive controls, showed a distinct increase in induced mutant colonies.

Under the experimental conditions reported, the test item did not induce gene mutations at the *Hprt* locus in V79 cells of the Chinese hamster. Therefore, cyprodinil is considered to be non-mutagenic in this *Hprt* assay (Wollny, 2017).

The potential for cyprodinil (purity 99.2%; lot/batch no. P.012011) dissolved in dimethyl sulfoxide (DMSO) to induce micronuclei in vitro was assessed using human lymphocytes in four independent experiments. In each experimental group, two parallel cultures were analysed. Per culture, 1000 binucleated cells were evaluated for cytogenetic damage. The highest applied concentration of the test substance in this study was 2000 µg/mL in experiment IA; however, precipitation was observed at 72.6 µg/mL (without S9) and 127 µg/mL (with S9). Precipitation was also observed in experiments IB (4-hour exposure, with and without S9) and IC (4-hour exposure, without S9), but not in experiment IIB (20-hour exposure, without S9).

Cytotoxicity was observed at high concentrations in all four independent experiments. In the absence and presence of S9, no relevant increase in the number of micronucleated cells was observed after treatment with cyprodinil.

Appropriate mutagens were used as positive controls. They induced statistically significant increases in cells with micronuclei.

Under the experimental conditions reported, the test substance did not induce micronuclei as determined by the in vitro micronucleus test in human lymphocytes. Cyprodinil is considered to be non-genotoxic in this in vitro micronucleus test when tested up to cytotoxic or precipitating concentrations (Naumann, 2017a).

2.3 Special studies

(a) Immunotoxicity

In an immunotoxicity study, cyprodinil (purity 99.2%; lot/batch no. P.012011) was given in the diet to female Crl:CD1 (ICR) mice (10 per group) at a concentration of 0, 500, 2000 or 5000 parts per million (ppm) (equal to 0, 104, 468 and 1245 mg/kg bw per day, respectively) for 28 days. Mice in a positive control group were administered cyclophosphamide at 50 mg/kg bw per day via intraperitoneal injection for 4 consecutive days (study days 24 through 27). The vehicle control and positive control groups were offered the basal diet on a comparable regimen as the cyprodinil-treated groups. Additionally, all mice received an intravenous injection of sheep red blood cells on study day 24, approximately 96 hours prior to the scheduled necropsy.

All animals survived to the scheduled necropsy. There were no test substance–related clinical observations. There were no treatment-related effects on body weight, feed consumption or macroscopic findings. There were no cyprodinil-related effects on absolute, adjusted or relative spleen or thymus weights. There were no effects on the specific activity or total activity of splenic immunoglobulin M antibody-forming cells to the T cell–dependent antigen sheep red blood cells that could be attributed to cyprodinil.

The NOAEL for immunotoxicity in mice was 5000 ppm (equal to 1245 mg/kg bw per day), the highest dose tested (Crittenden, 2010).

(b) Oral proof of exposure in mice

A study was performed to demonstrate proof of bone marrow exposure in the mouse after oral (gavage) administration of cyprodinil in a previously evaluated micronucleus study (Ceresa, 1990). Three male and three female mice of the CRL: CD-1 (ICR) strain were dosed once with cyprodinil (purity 99.2%; lot/batch no. P.012011) as a suspension in 0.5% carboxymethylcellulose at 2000 mg/kg bw using a dosing volume of 20 mL/kg bw.

No deaths or clinical signs were observed throughout the study. A single oral (gavage) administration of cyprodinil at 2000 mg/kg bw was well tolerated in all animals.

Proof of bone marrow exposure in the mouse following a single oral (gavage) administration of cyprodinil at 2000 mg/kg bw was demonstrated by detectable concentrations of cyprodinil in blood (Fincher, 2016).

(c) Phototoxicity

In the in vitro 3T3 NRU phototoxicity test, the phototoxic potential of cyprodinil (purity 99.2%; lot/batch no. P.012011) was determined in a cytotoxicity assay with the BALB/3T3 mouse fibroblast cell line. Cyprodinil was dissolved in 1% DMSO in Earle's balanced salt solution. BALB/3T3 cells were treated for 1 hour with different concentrations of the test solution at 37 ± 1 °C and for a further 50 minutes in the absence and presence of a non-toxic dose of ultraviolet A (UVA) light. One day after treatment, cytotoxicity was analysed as a measure of reduction of neutral red uptake and compared with the controls.

In this study, under the given conditions, cyprodinil showed a cytotoxic effect with and without irradiation. With irradiation, the viability of the cells was reduced to 10.8%, and without irradiation, to −0.1%. Based on this, the median effective concentrations (EC_{50} values) and the photoirritancy factor (PIF) were calculated as follows:

−UVA: EC_{50} = 21.17 µg/mL

+UVA: EC_{50} = 28.32 µg/mL

PIF = 0.748

Based on the criterion in Organisation for Economic Co-operation and Development (OECD) Test Guideline 432 (i.e. PIF < 2), cyprodinil is considered to produce no phototoxic effects (Lehmeier, 2014).

(d) Toxicity of metabolites

The genotoxicity of four plant or animal metabolites of cyprodinil was tested in vitro and/or in vivo. No evidence of genotoxicity was found (Table 3).

Table 3. Summary of the genotoxicity of cyprodinil metabolites

End-point	Test object	Concentrations/doses	Results	Purity / lot/batch no.	Reference
NOA422054 (4-cyclopropyl-6-hydroxymethyl-pyrimidin-2-ylamine; rotational crop metabolite)					
In vitro					
Gene mutation	Chinese hamster lung (V79) cells (*Hprt* locus)	*With/without S9* 0, 106.4, 212.9, 425.8, 851.5, 1 703.0 µg/mL	Negative	97% / MES489/1	Chang (2017a)

End-point	Test object	Concentrations/doses	Results	Purity / lot/batch no.	Reference
Micronucleus test	Human lymphocytes	*Without S9* Exp. 1A: 182, 318, 556, 973 µg/mL Exp. 1B: 391, 986, 1 419, 1 703 µg/mL Exp. 1C: 986, 1 183, 1 419, 1 703 µg/mL Exp. 2: 505, 757, 1 135, 1 703 µg/mL *With S9* Exp. 1A: 318, 556, 973, 1 703 µg/mL	Negative[a]	97% / MES 489/1	Naumann (2017b)
CGA321915 (4-cyclopropyl-6-methyl-pyrimidin-2-ol; rotational crop metabolite)					
In vitro					
Gene mutation	Chinese hamster lung (V79) cells (*Hprt* locus)	*Without S9* 96.8, 193.5, 387.0, 774.0, 1 548.0 µg/mL *With S9* 96.8, 193.5, 387.0, 774.0, 1 548.0 µg/mL	Negative	97% / MES 356/2	Chang (2017b)
Micronucleus test	Human lymphocytes	*With/without S9* 289, 505, 885, 1 548 µg/mL	Negative	97% / MES 356/2	Naumann (2017c)
CGA263208 (*N*-phenyl-guanidine; CA1139, Reg. No. 4182909[b]; rotational crop metabolite)					
In vitro					
Gene mutation	Chinese hamster lung (V79) cells (*Hprt* locus)	*Without S9* 650, 1 300, 1 733.3, 2 166.6, 2 600 µg/mL *With S9* 650, 1 300, 1 733.3, 2 166.6, 2 600 µg/mL	Negative	93.8% / WRS 1233/1	Chang (2017c)
In vivo					
Micronucleus assay[c]	Bone marrow cells of Wistar rats	Bone marrow cells collected after single administration 24-hour preparation interval: 125, 250, 500 mg/kg bw 48-hour preparation interval: 500 mg/kg bw	Negative	73.7% / L85-142	Dony (2015)
CGA304075 (4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamino)-phenol; rat metabolite, animal commodities)					
In vitro					
Gene mutation	Chinese hamster lung (V79) cells (*Hprt* locus)	*Without S9* Exp. I: 32.0, 42.7, 53.4, 64.0, 128.0 µg/mL	Negative	98% / MES500/1	Chang (2017d)

End-point	Test object	Concentrations/doses	Results	Purity / lot/batch no.	Reference
		Exp. II: 32.0, 64.0, 96.0, 128.0 µg/mL Exp. III: 12.5, 25.0, 50.0, 60.0 µg/mL *With S9* Exp. I: 16.0, 32.0, 64.0, 85.5 µg/mL Exp. II: 32.0, 64.0, 80.0, 96.0 µg/mL Exp. III: 30.0, 60.0, 70.0, 80.0 µg/mL			
Micronucleus assay	Human lymphocytes	*Without S9* Exp. IB: 35.1, 107, 207, 239 µg/mL Exp. IC: 99.3, 120, 145, 160 µg/mL *With S9* Exp. IIC: 136, 150, 182, 200 µg/mL	Negative	98% / MES500/1	Naumann (2017d)

bw: body weight; Exp.: Experiment; *Hprt*: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] In experiment 1A, in the absence of S9, one statistically significant increase in the number of micronucleated cells (1.20%) was observed after treatment with 973 µg/mL. The value also exceeded the 95% control limits of the historical solvent control data (0.07–1.15% micronucleated cells). This increase in micronuclei was not reproducible in experiments 1B and 1C, and therefore the increase in experiment 1A was considered to be a cytotoxicity-related artefact and not biologically relevant.

[b] Identified as a metabolite of BAS 605 F, pyrimethanil (Siebold, 2016).

[c] Proof of bone marrow exposure was demonstrated by Grauert & Kamp (2015).

Comments

Biochemical aspects

Cyprodinil was extensively metabolized in human and rat liver microsomes in vitro. No human-specific metabolites were detected (Thibaut, 2017).

Toxicological data

In three in vitro assays – a reverse mutation assay (Sokolowski, 2009), a gene mutation assay (Wollny, 2017) and a micronucleus test in human lymphocytes (Naumann, 2017a) – cyprodinil was not genotoxic. These results support the conclusion of the 2003 Meeting that cyprodinil is unlikely to be genotoxic.

In an immunotoxicity study using female mice given cyprodinil at a dietary concentration of 0, 500, 2000 or 5000 ppm (equal to 0, 104, 468 and 1245 mg/kg bw per day, respectively) for 28 days, the NOAEL for immunotoxicity was 5000 ppm (equal to 1245 mg/kg bw per day), the highest dose tested (Crittenden, 2010).

The Meeting concluded that cyprodinil is not immunotoxic.

In a study to support a previously evaluated in vivo micronucleus study (Ceresa, 1990), proof of bone marrow exposure in the mouse following a single oral (gavage) administration of cyprodinil at 2000 mg/kg bw was demonstrated by the detection of cyprodinil in blood (Fincher, 2016).

Cyprodinil showed no phototoxic effects (Lehmeier, 2014).

Toxicological data on metabolites

NOA422054 (4-cyclopropyl-6-hydroxymethyl-pyrimidin-2-ylamine; rotational crop metabolite)

NOA422054 was negative in a gene mutation assay using Chinese hamster lung cells (Chang, 2017a) and an in vitro micronucleus test using human lymphocytes (Naumann, 2017b).

The Meeting concluded that NOA422054 is unlikely to be genotoxic in vitro.

CGA321915 (4-cyclopropyl-6-methyl-pyrimidin-2-ol; rotational crop metabolite)

CGA321915 was negative in a gene mutation assay using Chinese hamster lung cells (Chang, 2017b) and an in vitro micronucleus test using human lymphocytes (Naumann, 2017c).

The Meeting concluded that CGA321915 is unlikely to be genotoxic in vitro.

CGA263208 (N-phenyl-guanidine; rotational crop metabolite)

CGA263208 was negative in a gene mutation assay using Chinese hamster lung cells (Chang, 2017c) and an in vivo micronucleus test in rats (Dony, 2015). Proof of bone marrow exposure was demonstrated (Grauert & Kamp, 2015).

The Meeting concluded that CGA263208 is unlikely to be genotoxic.

CGA304075 (4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamino)-phenol; rat metabolite, animal commodities)

CGA304075 was negative in a gene mutation assay using Chinese hamster lung cells (Chang, 2017d) and an in vitro micronucleus test using human lymphocytes (Naumann, 2017d).

The Meeting concluded that CGA304075 is unlikely to be genotoxic in vitro.

Toxicological evaluation

The Meeting concluded that it was not necessary to revise the ADI or establish an ARfD for cyprodinil.

The Meeting concluded that metabolites NOA422054, CGA321915, CGA263208 and CGA304075, which were evaluated by the present Meeting, and metabolites CGA249287 and CGA275535, which were evaluated by the 2003 Meeting, were unlikely to be genotoxic. Following the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015), the Meeting concluded that for chronic toxicity, these six metabolites could be assessed using the threshold of toxicological concern (TTC) approach. All six metabolites are categorized in Cramer class III, and therefore a TTC of 1.5 µg/kg bw per day applies.

Critical end-points for setting guidance values for exposure to cyprodinil and metabolites

Short-term studies of toxicity	
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Genotoxicity	
	No evidence of genotoxicity in vitro
Other toxicological studies	
Immunotoxicity NOAEL	1 245 mg/kg bw per day, highest dose tested (mouse)
Phototoxicity	No phototoxicity
Studies on metabolites	
NOA422054	No evidence of genotoxicity in vitro
CGA321915	No evidence of genotoxicity in vitro
CGA263208	No evidence of genotoxicity
CGA304075	No evidence of genotoxicity in vitro

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DICAMBA (addendum)

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Explanation

Dicamba (3,6-dichloro-2-methoxybenzoic acid) was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2010, when an acceptable daily intake (ADI) of 0–0.3 mg/kg body weight (bw) and an acute reference dose (ARfD) of 0.5 mg/kg bw were established (Annex 1, reference *121*).

The 2010 Meeting also assessed data on three metabolites of dicamba: DCSA (3,6-dichlorosalicylic acid), DCGA (3,6-dichlorogentisic acid) and 5-OH-dicamba (2,5-dichloro-3-hydroxy-6-methoxybenzoic acid). The 2010 Meeting concluded that DCSA and DCGA have toxicities similar to or lower than that of dicamba. Based on available data, the 2010 Meeting concluded that 5-OH-dicamba appeared to be of lower toxicity than the parent compound.

Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, dicamba was placed on the agenda of the present Meeting, which assessed additional toxicological information available since the last review.

The carcinogenicity phase of a previously evaluated chronic toxicity study of DCSA, which was conducted in compliance with good laboratory practice, was submitted to the present Meeting. In addition, the Meeting applied the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015) to the metabolites DCSA, DCGA and 5-OH-dicamba.

No additional information from a literature search was identified that complemented the toxicological information submitted for the current assessment.

Evaluation for acceptable intake

1. Toxicological studies on metabolites

1.1 DCSA (MON 52708; rat and soybean metabolite)

(a) Long-term studies of toxicity and carcinogenicity

A combined study of the chronic toxicity and carcinogenicity of DCSA was conducted using rats. The chronic toxicity phase (12 months) of the study was evaluated by the previous Meeting, and the no-observed-adverse-effect level (NOAEL) for chronic toxicity (12 months) was identified as 171.2 mg/kg bw per day, the highest dose tested (Annex 1, reference *121*).

All dosing groups initially consisted of 70 male and 70 female Sprague Dawley (Crl:CD[SD]) rats. For the carcinogenicity phase, groups of 50 male and 50 female rats received DCSA (purity 97.4–97.7%; lot no. GLP-0603-16958-T) in the diet at a concentration of 0, 10, 100, 300, 1000 or 3000 parts per million (ppm) (equal to 0, 0.5, 5.0, 14.6, 48.8 and 150.1 mg/kg bw per day for males and 0, 0.6, 6.1, 18.4, 60.9 and 181.5 mg/kg bw per day for females, respectively) for 24 months. The remaining 20 animals per group were assigned to the chronic toxicity phase (12 months) of the study.

There were no treatment-related changes in mortality, clinical signs or body weights in males or females in all treated groups up to 24 months. Microscopically, the incidences of pigmented macrophages in the mediastinal lymph node and macrophages in the spleen were statistically significantly increased in females at 3000 ppm (Table 1**)**. These increases were not considered treatment related, because the incidences of findings in rats at unscheduled death were comparable among all groups, including the control group, although the organs at termination in all but the highest-dose group were not examined. In addition, no related findings were observed in other lymphohaematopoietic organs or liver.

The incidence of hepatocellular adenomas in males at 3000 ppm was 6% (3/50), but it was not statistically significantly different from the control group incidence (2%; 1/50) (Table 1). All incidences of hepatocellular adenomas in all groups were within the historical control range (mean 2.10%; range 0–6% in 1050 male rats at the test facilities from January 2000 to June 2009), except for the incidence in males at 100 ppm (8%), but there was no dose–response relationship. Therefore, the incidence of hepatocellular adenoma was not considered to be treatment related.

In females, the incidences of endometrial stromal polyp were 2, 4, 4, 2, 9 and 6 at 0, 10, 100, 300, 1000 and 3000 ppm, respectively (Table 1). Protocol-specified statistical analysis (one-tailed Fisher's exact test with a Bonferroni correction to adjust the *P*-values for multiple comparisons) showed that the higher incidences of the polyps at 1000 and 3000 ppm were not statistically significant. The mean and range of historical control incidences of this finding in 1049 female rats at the test facility for 9 years (from January 2000 to June 2009) were 5.91% and 1.67–13.33%. Although the incidence at 1000 ppm was higher than the historical control range, all other incidences were within the historical control range. Endometrial stromal polyp is known to be a common benign tumour in rodents (Greaves, 2015). Endometrial stromal polyp has been reported following administration of a number of chemicals to rodents; however, no common mode of action has been suggested (Davis, 2012). In addition, no scientific or experimental evidence to date suggests that uterine stromal polyps in rodents are hormone sensitive, and therefore they appear to have limited relevance to endometrial polyps occurring in women. Taken together, the slightly higher incidences of endometrial stromal polyp at 1000 and 3000 ppm were not considered to be an adverse treatment-related effect, for the following reasons: 1) they are a common finding in aged rats; 2) there was a lack of statistical significance; 3) there was a lack of a clear dose–response relationship; 4) the incidence in the 3000 ppm group was within the range of historical control data at the facility where this study was conducted; and 5) there was a lack of progressive change leading to malignancy.

No treatment-related increase in the incidence of other tumours was observed.

The NOAEL for the chronic toxicity and carcinogenicity of DCSA was 3000 ppm (equal to 150.1 mg/kg bw per day), the highest dose tested (Kirkpatrick, 2011).

1.2 Comparison of toxicity profiles of dicamba and its metabolites

To facilitate the assessment of the metabolites DCSA, DCGA and 5-OH-dicamba using the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015), their toxicity profiles are compared in Table 2.

Table 1. Summary of histopathological findings in carcinogenicity study in rats

	Incidence of finding											
	Males						Females					
Finding	**0 ppm**	**10 ppm**	**100 ppm**	**300 ppm**	**1 000 ppm**	**3 000 ppm**	**0 ppm**	**10 ppm**	**100 ppm**	**300 ppm**	**1 000 ppm**	**3 000 ppm**
No. of rats examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
No. of rats at SN	*16*	*19*	*16*	*10*	*14*	*9*	*22*	*21*	*19*	*21*	*23*	*22*
Pigmented macrophage in the mediastinal lymph node												
All	10/50	–/29	–/34	–/38	–/36	13/49	19/46	–/28	–/29	–/27	–/29	30/45*
UD	5/34	3/29	4/33	8/37	4/36	9/40	8/26	10/28	10/28	9/26	16/27	10/23
SN	5/16	NE	0/1	0/1	NE	4/9	11/20	NE	NE	0/1	0/2	20/22*
Pigmented macrophage in the spleen												
All	14/50	–/35	–/37	–/41	–/37	15/50	23/50	–/29	–/34	–/31	–/28	35/50*
UD	12/34	4/30	13/34	13/40	9/36	14/41	17/28	15/29	18/31	16/29	14/27	19/28
SN	2/16	0/5	0/3	0/1	0/1	1/9	6/22	NE	0/3	0/2	0/1	16/22*
Liver, hepatocellular adenoma												
All	1/50	1/50	4/50	1/50	1/50	3/50	0/50	–/34	–/33	–/35	–/36	0/50
UD	0/34	0/31	2/34	0/40	1/36	3/41	0/28	0/29	0/31	1/29	0/27	0/28
SN	1/16	1/19	2/16	1/10	0/14	0/9	0/22	NE	NE	NE	NE	0/22
Stromal polyp in the uterus												
All							2/50	4/50	4/50	2/50	9/50	6/50
UD							1/28	1/29	3/31	1/29	3/27	2/28
SN							1/22	3/21	1/19	1/21	6/23	4/22

NE: not examined; ppm: parts per million; SN: scheduled necropsy; UD: unscheduled death; –: UD and SN not combined because of limited histopathological examination of organs with macroscopic findings in 10–1000 ppm groups; *: $P < 0.05$
Source: Kirkpatrick (2011)

Table 2. Comparison of the toxicity profiles of dicamba and its metabolites DCSA, DCGA and 5-OH-dicamba

	LOAEL/NOAEL			
Study end-point	**Dicamba[a]**	**DCSA[b]**	**DCGA[a]**	**5-OH-Dicamba[a]**
Genotoxicity	Negative in Ames test; negative in vivo	Negative in Ames test; negative in vivo	Negative in Ames test; negative in vivo	Negative in Ames test; negative in vivo
Oral LD_{50} (rats)	1 600 mg/kg bw	2 641 mg/kg bw	1 460 mg/kg bw	>2 000 mg/kg bw
Toxicity profile	Mild effects, including reduced body weight gain, haematology, clinical chemistry or clinical signs	Mild effects, including reduced body weight gain	Mild effects, including reduced body weight gain or clinical signs	No effects, but limited database
Short-term toxicity (rats)	1 000/479.3 mg/kg bw per day (13 weeks)	436/222 mg/kg bw per day[c] (13 weeks)	956/474 mg/kg bw per day (4 weeks)	–/25 mg/kg bw per day (13 weeks)
Short-term toxicity (dogs)	300/52 mg/kg bw per day (13 and 52 weeks combined)	150/50 mg/kg bw per day (13 weeks)	No data	–/6.25 mg/kg bw per day (13 weeks)
Long-term toxicity (rats)	–/107 mg/kg bw per day	–/150.1 mg/kg bw per day	No data	No data
Carcinogenicity (rats)	107/11 mg/kg bw per day (equivocal)	–/150.1 mg/kg bw per day	No data	No data
Reproductive toxicity (rats)				
Parental toxicity	347/105 mg/kg bw per day	323/37 mg/kg bw per day	No data	No data
Offspring toxicity	105/35.1 mg/kg bw per day	323/37 mg/kg bw per day		
Reproductive toxicity	–/347 mg/kg bw per day	–/323 mg/kg bw per day		
Developmental toxicity (rats)			*Pilot study only*	
Maternal toxicity	400/160 mg/kg bw per day	–/100 mg/kg bw per day	200/50 mg/kg bw per day	No data
Embryo/fetal toxicity	–/400 mg/kg bw per day	–/100 mg/kg bw per day	–/1 000 mg/kg bw per day	No data
Developmental toxicity (rabbits)				
Maternal toxicity	150/30 mg/kg bw per day	65/25 mg/kg bw per day	No data	No data
Embryo/fetal toxicity	–/300 mg/kg bw per day	–/65 mg/kg bw per day	No data	No data

–: no LOAEL, NOAEL is the highest dose tested; bw: body weight; LD_{50}: median lethal dose; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level

[a] *Source*: Annex 1, reference *121*.

[b] *Source*: Annex 1, reference *121*, except for the long-term study (Kirkpatrick, 2011).

[c] The 2010 Meeting identified LOAEL/NOAEL values of 362/195 mg/kg bw per day based on the male rat dietary intake values. However, as the effects were seen only in females, the LOAEL/NOAEL values for the female rat are given in the above table.

Comments

Toxicological data on metabolites and/or degradates

DCSA (rat, crop and livestock metabolite and environmental degradate)

In a carcinogenicity study in rats treated with DCSA in the diet at a concentration of 0, 10, 100, 300, 1000 or 3000 ppm (equal to 0, 0.5, 5.0, 14.6, 48.8 and 150.1 mg/kg bw per day for males and 0, 0.6, 6.1, 18.4, 60.9 and 181.5 mg/kg bw per day for females, respectively) for 24 months, the NOAEL for toxicity was 3000 ppm (equal to 150.1 mg/kg bw per day), the highest dose tested. No carcinogenicity was observed (Kirkpatrick, 2011).

The Meeting concluded that DCSA is not carcinogenic in rats.

In view of the lack of genotoxicity of DCSA in vivo (Annex 1, reference *121*) and the absence of carcinogenicity in rats, the Meeting concluded that DCSA is unlikely to pose a carcinogenic risk to humans.

DCGA (crop metabolite and environmental degradate)

The 2010 Meeting considered that the toxicity of DCGA was similar to or lower than that of dicamba. No new toxicological data were submitted on DCGA.

5-OH-Dicamba (rat metabolite)

The 2010 Meeting considered that, on the basis of the available information, 5-OH-dicamba appears to be of lower toxicity than dicamba. No new toxicological data were submitted on 5-OH-dicamba.

Toxicological evaluation

The Meeting concluded that it was not necessary to revise the ADI or ARfD established for dicamba by the 2010 Meeting.

The Meeting concluded that DCSA and DCGA are toxicologically relevant and likely to be of similar or lower toxicity compared with the parent compound. Following the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015), the Meeting concluded that these metabolites would be covered by the ADI and ARfD for dicamba.

The Meeting concluded that 5-OH-dicamba may be toxicologically relevant and appears to be of lower toxicity than the parent compound based on the limited database and the metabolite's structural relationship to the parent compound, likely leading to more rapid excretion. Following the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015), the Meeting concluded that 5-OH-dicamba would be covered by the ADI and ARfD for dicamba. Owing to the limited database, the Meeting was unable to conclude that 5-OH-dicamba was of no concern.

The ADI of 0–0.3 mg/kg bw applies to dicamba, DCSA, DCGA and 5-OH-dicamba, expressed as dicamba.

The ARfD of 0.5 mg/kg bw applies to dicamba, DCSA, DCGA and 5-OH-dicamba, expressed as dicamba.

Acceptable daily intake (ADI) (applies to dicamba, DCSA, DCGA and 5-OH-dicamba, expressed as dicamba)

0–0.3 mg/kg bw

Acute reference dose (ARfD) (applies to dicamba, DCSA, DCGA and 5-OH-dicamba, expressed as dicamba)

0.5 mg/kg bw

Critical end-points for setting guidance values for exposure to dicamba metabolites

Studies on metabolites	
DCSA	
Two-year toxicity study	NOAEL for toxicity 150.1 mg/kg bw per day, highest dose tested (rat)
	No evidence of carcinogenicity[a] (rat)

[a] In view of the lack of genotoxicity of DCSA in vivo and the absence of carcinogenicity in rats, DCSA is unlikely to pose a carcinogenic risk to humans.

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MESOTRIONE (addendum)

First draft prepared by
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Explanation

Mesotrione (2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione) was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2014, when an acceptable daily intake (ADI) of 0–0.5 mg/kg body weight (bw) was established. The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for mesotrione (Annex 1, reference *133*).

The 2014 Meeting also assessed data on two metabolites of mesotrione: AMBA (2-amino-4-methylsulfonylbenzoic acid) and MNBA (2-nitro-4-methylsulfonylbenzoic acid). On the basis of the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015), the 2014 Meeting concluded that these two metabolites were unlikely to be a safety concern (Annex 1, reference *133*).

Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, mesotrione was placed on the agenda of the present Meeting, which assessed additional toxicological information available since the last review.

Several toxicological studies on the two metabolites of mesotrione, AMBA and MNBA, were submitted to the present Meeting, including a study on systemic exposure to AMBA from MNBA, two-generation reproductive toxicity and developmental toxicity studies with MNBA, and an in vivo micronucleus assay with AMBA. No new information on mesotrione, the parent compound, was submitted.

All critical studies contained statements of compliance with good laboratory practice and were conducted in accordance with relevant national or international test guidelines, unless otherwise specified. No additional information from a literature search was identified that complemented the toxicological information submitted for the current assessment.

Evaluation for acceptable intake

1. Biochemical aspects of metabolites

1.1 Systemic exposure to AMBA and MNBA

AMBA is a metabolite of MNBA that is postulated to be predominantly formed in the gut from unabsorbed MNBA by intestinal microflora and is then absorbed into the systemic circulation. To investigate and quantify the systemic exposure to AMBA in blood and plasma following exposure to MNBA, female Han Wistar rats were administered a single oral gavage dose of MNBA (purity 99.8%; batch no. 694472) in 1% weight per volume (w/v) aqueous carboxymethylcellulose at 75 mg/kg bw. A blood sampling schedule (0.25–72 hours), capable of fully characterizing the systemic exposure to both MNBA and AMBA in the rat, was defined by a preliminary pharmacokinetics study. A temporary tail vein cannula and use of volumetric absorption microsampling allowed an appropriate number of 10 µL serial blood samples to be taken from individual animals to characterize the concentration–time profiles of both MNBA and AMBA. Larger volumes (approximately 100 µL) of blood were removed at 0.5, 10, 24 and 48 hours to allow for plasma analysis. Concentrations of MNBA and AMBA in blood and plasma were determined by liquid chromatography with tandem mass spectrometry (LC-MS/MS), using validated bioanalytical methods. To obtain more consistent data and simplify their interpretation, the systemic exposure (area under the concentration–time curve [AUC]) to each analyte in rat blood was derived from MNBA and AMBA concentration data up to 24 hours post-dosing.

The AUCs of MNBA and AMBA and the ratios of their concentrations in blood and plasma are summarized in Table 1.

Table 1. Systemic exposure to MNBA and AMBA in rats treated orally with MNBA at a dose of 75 mg/kg bw

Parameter		MNBA		AMBA	
$AUC_{0–24\ h}$ (ng·h/mL)					
		Replicate A[a]	Replicate B[b]	Replicate A[a]	Replicate B[b]
	No. of rats	*5*	*5*	*5*	*5*
	Mean ± SD	3 492 ± 902	4 050 ± 343	9 378 ± 6 089	6 784 ± 1 382
Plasma concentration (ng/mL)					
Nominal time after dosing (hours)	0.5	2 355 ± 860		12.2 ± 3.54	
	10	241 ± 223		1 244 ± 1 043	
	24	NC		139 ± 201	
	48	NC		NC	
Blood:plasma concentration ratio					
Nominal time after dosing (hours)	0.5	0.71 ± 0.33		1.14 ± 0.72	
	10	0.90 ± 0.82		0.88 ± 0.20	
	24	NC		0.80 ± 0.13	
	48	NC		NC	

AMBA: 2-amino-4-methylsulfonylbenzoic acid; $AUC_{0–24\ h}$: area under the concentration–time curve from 0 to 24 hours; bw: body weight; MNBA: 2-nitro-4-methylsulfonylbenzoic acid; NC: not calculable, as no plasma data were available for these time points

[a] Results generated from data within the recognized stability period.

[b] Results generated from data 16 days beyond the recognized stability period. However, as the concentrations of MNBA in rat blood, determined by analysis of replicates A and B, were generally similar, the calculation of the $AUC_{0–24\ h}$ from the replicate B samples was justified and suggests that MNBA is stable in rat blood for up to 24 days.

Source: Punler (2017)

Following single oral gavage administration of MNBA to female Han Wistar rats at a dose of 75 mg/kg bw, the concentration–time profiles of both MNBA and AMBA, although variable, were sufficiently well characterized to confirm the systemic exposure to AMBA, with systemic concentrations of AMBA being reported from 0.25 to 72 hours post-dosing. Comparison of the systemic exposure quantified in terms of blood $AUC_{0-24\ h}$ shows a 2- to 3-fold higher exposure to AMBA compared with MNBA over the 24-hour period following dosing with MNBA. Concentrations of MNBA and AMBA in rat plasma were similar to those observed in blood, indicating that both compounds distribute into red blood cells, but are also still freely available in plasma (Punler, 2017).

2. Toxicological studies on metabolites

2.1 AMBA

(a) In vivo genotoxicity

AMBA was assessed for its potential to cause damage to chromosomes or cell division apparatus or to cause cell cycle interference, leading to micronucleus formation in polychromatic erythrocytes in the bone marrow of young adult rats. In all phases, the dosing of the vehicle and test item was by oral (gavage in 1% aqueous carboxymethylcellulose) administration twice, approximately 24 hours apart.

In the range-finding study, three male and three female Crl:WI(Han) rats were given a single dose of AMBA (purity 98.6%; lot/batch no. 924777) at 2000 mg/kg bw, the limit dose, by gavage. The maximum tolerated dose was confirmed to be greater than the limit dose of 2000 mg/kg bw in both sexes, and, as there was no difference in toxicity between the sexes, the main study was conducted in males only. Proof of bone marrow exposure to AMBA was confirmed as part of the range-finding study via LC-MS/MS analysis using the whole blood of animals given AMBA.

For the main study, six male Crl:WI(Han) rats were dosed with AMBA at 0, 500, 1000 or 2000 mg/kg bw by gavage. In a positive control group, six male rats were given a single 15 mg/kg bw dose of cyclophosphamide monohydrate by gavage. Bone marrow was harvested from all range-finding and main study animals approximately 24 hours after the final dose administration, and smears were prepared. The stained slides prepared for the main study were coded, 6000 polychromatic erythrocytes per animal were scored for the presence of micronuclei, and the group frequencies were statistically analysed.

There were no statistically significant increases in micronucleus frequency in male rats administered AMBA at any dose.

AMBA gave negative results in the rat bone marrow micronucleus assay in vivo (Dunton, 2016).

2.2 MNBA

(a) Reproductive toxicity

In a two-generation reproductive toxicity study, Crl:WI(Han) rats (30 of each sex per group) were given MNBA (purity 99.8%; lot/batch no. 694472) in 1% (w/v) aqueous carboxymethylcellulose vehicle at a dose of 0, 100, 300 or 1000 mg/kg bw per day via oral gavage. Evaluations of the male and female rat reproductive systems included gonadal function, estrous cycle, mating behaviour, conception, gestation, parturition, lactation and weaning, and the growth and development of the offspring over two successive generations.

There were no treatment-related changes in clinical signs, body weight or feed consumption in either generation. Kidney weights (absolute and relative to body weight) were increased at 1000 mg/kg bw per day in male rats in the parental generation; however, the increase was not statistically significant. At 1000 mg/kg bw per day, organ weights of bilateral kidneys were statistically significantly increased in males and females of the first filial generation. However, their magnitudes were slight (males: 9%

and 8% for left and right absolute kidney weights; 8% and 6% for left and right relative kidney weights; females: 6% and 5% for left and right absolute kidney weights). No corresponding histopathological changes were observed in the kidney in either generation. Therefore, the slight increases in kidney weights were not considered to be toxicologically relevant. There were no effects on reproductive function or performance, mating behaviour, conception or pup development at any dose. In addition, there were no macroscopic findings or histopathological changes related to the test substance at any dose, as evaluated in either adults or offspring.

The no-observed-adverse-effect level (NOAEL) for parental, offspring and reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested (Gilmore, 2016).

(b) Developmental toxicity

In a developmental toxicity study, female Crl:WI(Han) rats (22 per group) were given MNBA (purity 99.8%; lot/batch no. SMO3C0689) suspended in 1% (w/v) aqueous carboxymethylcellulose vehicle at a dose of 0, 100, 300 or 1000 mg/kg bw per day by gavage once daily from day 6 to day 19 of gestation. During the study, clinical observations were recorded, and body weights and feed intake were measured. On day 20 of gestation, the dams were killed, and the live fetuses were removed from the uterus and weighed. The sex of the fetuses was determined, and the fetuses were examined for external, visceral, skeletal and cartilaginous abnormalities. Placental and gravid uterine weights were also recorded.

There were no deaths during the study, and no clinical observations considered to be related to MNBA treatment were noted. Overall mean body weight gain was slightly lower (within 10%) at 1000 mg/kg bw per day, but there was no statistical significance or dose–response relationship. Feed intake at 1000 mg/kg bw per day was statistically significantly decreased only from gestation day 15 to gestation day 18, but the decrease was very slight in magnitude (25.1, 23.5, 22.7 and 23.2 g at 0, 100, 300 and 1000 mg/kg bw per day, respectively). As this slight decrease in feed intake had minimal effects on body weight, it was not considered to be adverse. There were six non-pregnant females, two in each of the groups given 100, 300 or 1000 mg/kg bw per day. The uterine and fetal data were unaffected by the treatment. There was no adverse effect of MNBA on the incidence of major or minor external, visceral, skeletal or cartilaginous fetal abnormalities or variations.

The NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Pottle, 2016).

2.3 Comparison of toxicity of metabolites with toxicity of parent compound

The toxicity profiles for the metabolites AMBA and MNBA and mesotrione are compared in Table 2.

Table 2. Comparison of toxicity profiles for mesotrione and its metabolites AMBA and MNBA

	LOAEL/NOAEL		
Study end-point	**Mesotrione**	**AMBA**	**MNBA**
Acute toxicity (rats)	LD_{50} >5 000 mg/kg bw	LD_{50} >5 000 mg/kg bw	LD_{50} >5 000 mg/kg bw
Primary toxicity profiles	Effects on eye, kidney, liver and thyroid due to inhibition of 4-HPPD	Covered by in situ production in MNBA studies	Minor clinical chemistry changes
Ninety-day toxicity (rat)	0.63/0.41 mg/kg bw per day	ND	263.7/50.6 mg/kg bw per day

Study end-point	LOAEL/NOAEL		
	Mesotrione	**AMBA**	**MNBA**
Genotoxicity	Negative in in vivo micronucleus assay	Negative in in vivo micronucleus assay	Negative in in vitro and in vivo assays, including micronucleus assay
Reproductive toxicity (rat)			
Parental toxicity	1.1/0.3 mg/kg bw per day	ND	–/1 000 mg/kg bw per day
Reproductive toxicity	297/11.7 mg/kg bw per day	ND	–/1 000 mg/kg bw per day
Offspring toxicity	0.3/– mg/kg bw per day	ND	–/1 000 mg/kg bw per day
Developmental toxicity (rat)			
Maternal toxicity	100/– mg/kg bw per day	ND	–/1 000 mg/kg bw per day
Embryo/fetal toxicity	100/– mg/kg bw per day	ND	–1 000 mg/kg bw per day

/–: no NOAEL (i.e. effects observed at all doses); –/: no LOAEL (i.e. NOAEL is the highest dose tested); 4-HPPD: 4-hydroxyphenylpyruvate dioxygenase; AMBA: 2-amino-4-methylsulfonylbenzoic acid; bw: body weight; LD_{50}: median lethal dose; LOAEL: lowest-observed-adverse-effect level; MNBA: 2-nitro-4-methylsulfonylbenzoic acid; ND: no data; NOAEL: no-observed-adverse-effect level

Source: Annex 1, reference *133*, and current monograph

Based on these toxicity profiles, it is apparent that these two metabolites are of much lower toxicity than the parent compound, consistent with their very weak 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) inhibiting activities. The Meeting noted that although there were no repeated-dose studies on AMBA, its toxicity was considered to be addressed by studies with MNBA, owing to the high levels of AMBA detected following dosing with MNBA.

Comments

Biochemical aspects of metabolites

Systemic exposure to AMBA was confirmed following a single oral gavage administration of MNBA to rats at 75 mg/kg bw. Exposure to AMBA was 2- to 3-fold higher than exposure to MNBA based on blood $AUC_{0–24\,h}$. Both metabolites were detected in red blood cells and plasma (Punler, 2017).

Toxicological data on metabolites

AMBA (plant, livestock and rat metabolite)

AMBA was negative in an in vivo micronucleus assay in rats. Taken together with the results of the in vitro genotoxicity tests evaluated by the 2014 Meeting (Annex 1, reference *133*), the current Meeting concluded that AMBA is unlikely to be genotoxic in vivo.

MNBA (plant, livestock and rat metabolite)

In a two-generation reproductive toxicity study in rats treated with MNBA at 0, 100, 300 or 1000 mg/kg bw per day via oral gavage, the NOAEL for parental, offspring and reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested (Gilmore, 2016).

In a developmental toxicity study in rats treated with MNBA at 0, 100, 300 or 1000 mg/kg bw per day by gavage from day 6 to day 19 of gestation, the NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Pottle, 2016).

Toxicological evaluation

The Meeting concluded that it was not necessary to revise the ADI or establish an ARfD for mesotrione. In addition, the Meeting confirmed the previous conclusion by the 2014 JMPR that MNBA and AMBA are unlikely to be a safety concern.

Critical end-points for setting guidance values for exposure to mesotrione metabolites

Studies on metabolites	
MNBA	
Systemic exposure to MNBA and AMBA in the rat following oral exposure to MNBA	Administration of MNBA leads to systemic exposure to both MNBA and AMBA
Two-generation reproductive toxicity study	
Parental toxicity NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Offspring toxicity NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Reproductive toxicity NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Developmental toxicity study	
Maternal toxicity NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Embryo/fetal toxicity NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
AMBA	
In vivo rat micronucleus assay	No evidence of genotoxicity

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METAFLUMIZONE (addendum)

First draft prepared by
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Explanation

Metaflumizone ((*EZ*)-2′-[2-(4-cyanophenyl)-1-(α,α,α-trifluoro-*m*-tolyl)ethylidene]-4-(trifluoromethoxy)carbanilohydrazide) was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2009, when an acceptable daily intake (ADI) of 0–0.1 mg/kg body weight (bw) was established. The 2009 Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for metaflumizone (Annex 1, reference *118*).

The 2009 Meeting also assessed data on two metabolites of metaflumizone: M320I23 (4-{5-hydroxy-3-oxo-4-[4-(trifluoromethoxy)phenyl]-6-[3-(trifluoromethyl)phenyl]-2,3,4,5-tetrahydro-1,2,4-triazin-5-yl}benzonitrile) and M320I29 (*m*-trifluoromethyl benzoic acid) (Annex 1, reference *118*).

Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, metaflumizone was placed on the agenda of the present Meeting, which assessed additional toxicological information available since the last review. The Meeting also applied the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015) to previously evaluated metabolites as well as those considered at the present meeting.

Several toxicological studies on metaflumizone were submitted to the present Meeting, including further information on previously evaluated short-term and developmental toxicity studies, in vitro genotoxicity studies, two immunotoxicity studies and studies to assess androgenic and estrogenic effects of metaflumizone. In addition, studies on acute toxicity, dermal and eye irritation and in vitro genotoxicity for metabolite M320I04 (4-{2-oxo-2-[3-(trifluoromethyl)phenyl]ethyl}benzonitrile) and on in vitro and in vivo genotoxicity for metabolite M320I06 (4-cyanobenzoic acid) were submitted.

All critical studies contained statements of compliance with good laboratory practice and were conducted in accordance with relevant national or international test guidelines, unless otherwise specified. No additional information from a literature search was identified that complemented the toxicological information submitted for the current assessment.

Evaluation for acceptable intake

1. Toxicological studies

1.1 Short-term studies of toxicity

An additional amendment to the 1-year toxicity study in dogs, which was evaluated by the previous Meeting (Annex 1, reference *118*), was conducted to characterize the brown pigments found in the tubular epithelium of kidneys in that dog study using special staining. For this characterization, one male from the control group and one male from the high-dose (30 mg/kg bw per day) group were selected. The kidneys of selected animals with different grades of brown pigmentation in the tubular epithelium were stained as follows: Perl's stain (Berlin blue) for haemosiderin, Hall method for bilirubin and autofluorescence for lipofuscin.

The brown pigment in the tubular epithelium in the kidney was judged to be cytoplasmic lipofuscin based on fluorescence microscopy characterization, which confirms the conclusion of the previous meeting (Annex 1, reference *188*). The pigments were negative for haemosiderin and bilirubin (Kaspers, 2008).

1.2 Genotoxicity

(a) In vitro

In an Ames test, *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2uvrA were incubated with metaflumizone (purity 97.2%; batch no. 6335H019GB) dissolved in dimethyl sulfoxide (DMSO) in the presence or absence of metabolic activation (phenobarbital/β-naphthoflavone-induced rat liver S9 fraction). In a standard plate test, all bacteria strains were tested with doses ranging from 20 to 5000 µg/plate. In a preincubation test, *Salmonella* strains were tested with doses ranging from 1.6 to 400 µg/plate without S9 and from 8 to 2000 µg/plate with S9. A preincubation test with *E. coli* WP2uvrA was conducted at concentrations ranging from 20 to 5000 µg/plate.

Precipitation of the test substance was found from about 100 µg/plate onward without S9 and from about 500 µg/plate onward with S9. A cytotoxic effect was observed from about 100 µg/plate onward, depending on the strain and test conditions. A relevant increase in the number of his^+ or trp^+ revertants was not observed in the standard plate or the preincubation test either with or without S9.

This study indicated that metaflumizone was not mutagenic in the *S. typhimurium*/*E. coli* reverse mutation assay either with or without metabolic activation (Schulz & Landsiedel, 2009a).

Another Ames test was conducted using metaflumizone (nominal active ingredient concentration: 50 g/L) containing 8 g/kg Reg. No. 5051812/5051844 (identity not provided) and 1 g/kg Reg. No. 4110910 (identity not provided) dissolved in DMSO. *Salmonella* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2uvrA were incubated with metaflumizone containing 8 g/kg Reg. No. 5051812/5051844 and 1 g/kg Reg. No. 4110910 (composition: batch no. 306PB004GB DMSO1 – 45.5 g/L metaflumizone in DMSO; batch no. 306PY004GB DMSO2 – 51.6 g/L metaflumizone in DMSO; both batches contained 0.02 g/L Reg. No. 5051844, 0.42 g/L Reg. No. 5051812 and 0.06 g/L Reg. No. 4110910 in DMSO) with or without S9. *Salmonella* strains and *E. coli* WP2uvrA were tested in a standard plate test and a preincubation test at various concentrations.

Precipitation was noted from about 333 µg/plate onward, depending on the test conditions. A cytotoxic effect was observed from about 100 µg/plate onward, depending on the strain and test conditions. A relevant increase in the number of his^+ or trp^+ revertants was not observed in the standard plate or the preincubation test either with or without S9. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system. The vehicle controls induced a number of revertants that was comparable with historical control data for each strain, thus demonstrating the validity of the test performance.

The results indicated that metaflumizone containing Reg. No. 5051812/5051844 and Reg. No. 4110910 was not mutagenic in the *S. typhimurium*/*E. coli* reverse mutation assay in the absence or presence of metabolic activation under the experimental conditions of this study (Woitkowiak, 2015).

Both studies are summarized in Table 1.

Table 1. Summary of in vitro genotoxicity studies with metaflumizone or metaflumizone containing Reg. No. 5051812/5051844 and Reg. No. 4110910

End-point	Test object	Concentrations	Purity	Results	Reference
Reverse mutation	*S. typhimurium* (TA98, TA100, TA1535, TA1537)	Exp. 1 with/without S9, 20–5 000 µg/plate Exp. 2 without S9, 250–400 µg/plate / with S9, 125–2 000 µg/plate Exp. 3[a] without S9, 1.6–400 µg/plate / with S9, 8–2 000 µg/plate	97.2%	Negative	Schulz & Landsiedel (2009a)
	E. coli (WP2uvrA)	Exp. 1 & 3[a]: 20–5 000 µg/plate	97.2%	Negative	
Reverse mutation	*S. typhimurium* (TA98, TA100, TA1535, TA1537)	Exp. 1 without S9: 1.0–1 000 µg/plate / with S9, 3.3–2 500 µg/plate Exp. 2 without S9, 0–333 µg/plate / with S9, 1.0–1 000 µg/plate	45.5 or 51.6 g/L DMSO with Reg. No. 5051812, Reg. No. 5051844 and Reg. No. 4110910	Negative	Woitkowiak (2015)
	E. coli (WP2uvrA)	Exp. 1 & 2: with/without S9: 10–5 000 µg/plate		Negative	

DMSO: dimethyl sulfoxide; S9: 9000 × *g* supernatant fraction from rat liver homogenate
[a] Preincubation test.

1.3 Developmental toxicity

Additional information on absent subclavian artery, which was increased in incidence at 300 mg/kg bw per day in the previously evaluated developmental toxicity study in rabbits (Annex 1, reference *118*), was provided to the present Meeting. This new submission (Melching-Kollmuss, 2015; Schneider, 2015; DeSesso & Williams, 2017) provided no data that would change the 2009 Meeting's conclusion that the fetal and litter incidences of absent subclavian artery in rabbits were similar to the incidences in the historical controls for this finding in rabbits. The Meeting confirmed the conclusion of the 2009 Meeting that metaflumizone is not teratogenic.

1.4 Special studies

(a) Immunotoxicity

Metaflumizone (purity 95.8%; batch no. PF-01-02-Ve-1-3) in 1% aqueous methylcellulose was administered to groups of 10 female Wistar rats by gavage at a dose of 0, 15, 40 or 75 mg/kg bw per day for 28 days to investigate the immunotoxic effects of metaflumizone. Cyclophosphamide monohydrate was used as the positive control. On day 22, all animals received two intraperitoneal injections (0.25 mL each) of a sheep red blood cell suspension containing 4×10^8 cells/mL. On day 28, blood samples were obtained and analysed by enzyme-linked immunosorbent assay for anti–sheep red blood cell immunoglobulin M. In addition, rats were euthanized and subjected to a gross pathological examination, and weights of the adrenals, liver, thymus and spleen were determined. Selected organs were preserved but not examined.

No treatment-related mortality or clinical signs of toxicity occurred. Body weight gain and feed consumption were decreased at 75 mg/kg bw per day. Mean body weight gain was reduced during the second half of the treatment, statistically significantly between study days 17 and 28. There was no treatment-related effect on immunotoxicity parameters. No treatment-related gross necropsy findings were observed.

The no-observed-adverse-effect level (NOAEL) for systemic toxicity was 40 mg/kg bw per day, based on the decreased body weight gain and feed consumption at 75 mg/kg bw per day. The NOAEL for immunotoxicity was 75 mg/kg bw per day, the highest dose tested (Buesen et al., 2011).

In a second immunotoxicity study, metaflumizone (purity 95.8%; batch no. PF-01-02-Ve-1-3) in 1% aqueous carboxymethylcellulose was administered to groups of 10 female Wistar rats by gavage at a dose of 0, 15, 40 or 75 mg/kg bw per day for 28 days. Anti-asialo GM1 was used as the positive control. On day 28, rats were euthanized and subjected to a gross pathological examination, and weights of the adrenals, liver, thymus, lymph nodes (mesenteric and mandibular) and spleen were determined. Additionally, for spleen, innate immunity was measured using the natural killer cell assay.

No treatment-related mortality or clinical signs of toxicity occurred. Body weights were slightly (approximately 10%), but statistically significantly, reduced at 75 mg/kg bw per day throughout the study. Feed consumption was slightly (approximately 15%) decreased during the second half of the treatment. At 40 and 75 mg/kg bw per day, a slight (approximately 10%), but statistically significant, increase in relative spleen weight was observed. No corresponding findings were detected at necropsy in these groups. No treatment-related gross necropsy findings were observed. The increase in relative spleen weight was not considered to be biologically relevant because the increase would not be expected with immunosuppression. There were no treatment-related changes indicating immunotoxicity, such as effects on natural killer cell function.

The NOAEL for systemic toxicity was 40 mg/kg bw per day, based on reduced body weights and lower feed consumption at 75 mg/kg bw per day. The NOAEL for immunotoxicity was 75 mg/kg bw per day, the highest dose tested (Crittenden, 2011).

(b) Androgenic/estrogenic effects

Metaflumizone (purity 95.8%; batch no. PF-01-02-Ve-1-3) was administered to assess androgenic and/or anti-androgenic activity using the yeast androgen screening assay with the hAR yeast strain. Two independent experiments were carried out. Vehicle (DMSO) and positive controls for androgenic effects (5α-dihydrotestosterone at concentrations ranging from 10 pmol/L to 1 µmol/L) and anti-androgenic effects (5α-dihydrotestosterone at 5 nmol/L combined with hydroxyflutamide at 10 µmol/L) were included in the experiment. Metaflumizone was tested at concentrations ranging from 100 pmol/L to 100 µmol/L. Hydroxyflutamide was used as an anti-androgenic compound.

Metaflumizone showed no androgenic activity in comparison with 5α-dihydrotestosterone. Metaflumizone showed no anti-androgenic activity when compared with the effects induced by

hydroxyflutamide. Thus, under the conditions of this study, metaflumizone did not exert androgenic or anti-androgenic effects in the yeast androgen screening assay using the hAR yeast strain (Woitkowiak, 2011a).

Metaflumizone (purity 95.8%; batch no. PF-01-02-Ve-1-3) was administered to assess estrogenic and/or anti-estrogenic activity using the yeast estrogen screening assay with the hERα yeast strain. Two independent experiments were carried out. Vehicle (DMSO) and positive controls for estrogenic effects (17β-estradiol at concentrations ranging from 1 pmol/L to 1 µmol/L) and anti-estrogenic effects (17β-estradiol at 1 nmol/L combined with 4-hydroxytamoxifen at 1 µmol/L) were included in the experiment. Metaflumizone was tested at concentrations ranging from 100 pmol/L to 100 µmol/L. 4-Hydroxytamoxifen was used as an anti-estrogenic compound.

Metaflumizone showed no estrogenic activity in comparison with 17β-estradiol. Metaflumizone showed no anti-estrogenic activity when compared with the effects induced by 4-hydroxytamoxifen. Thus, under the conditions of this study, metaflumizone did not exert estrogenic or anti-estrogenic effects in the yeast estrogen screening assay using the hERα yeast strain (Woitkowiak, 2011b).

2. Toxicological studies on metabolites

2.1 M320I04 (4-{2-oxo-2-[3-(trifluoromethyl)phenyl]ethyl}benzonitrile; plant metabolite)

(a) Acute toxicity

Lethal dose studies and studies on dermal and ocular irritation are summarized in Table 2.

Table 2. Acute toxicity studies with metaflumizone metabolite M320I04

Study	Species/strain	Sex	Route	Purity/lot no.	Results	Reference
Acute toxicity	Rat/NS	M/F	Oral	99.3% / LJ30551/65	LD_{50} > 2 000 mg/kg bw	Gamer (2002)
Skin irritation	Rabbit/NZW	NS	Dermal	99.3% / LJ30551/65	No irritation	Remmele (2002a)
Eye irritation	Rabbit/NZW	NS	Ocular	99.3% / LJ30551/65	No irritation	Remmele (2002b)

bw: body weight; F: females; LD_{50}: median lethal dose; M: males; NS: not specified; NZW: New Zealand white

(b) Genotoxicity

A reverse mutation assay using metabolite M320I04 indicates that M320I04 is not mutagenic (Table 3).

2.2 M320I06 (4-cyanobenzoic acid; soil, plant and livestock metabolite; Reg. No. 121464)

(a) Genotoxicity

Genotoxicity studies with metabolite M320I06 are summarized in Table 4. These genotoxicity studies indicate that metabolite M320I06 is not genotoxic in vitro or in vivo.

Table 3. Genotoxicity study with metaflumizone metabolite M320I04

End-point	Test object	Concentrations	Purity/lot no.	Results	Reference
In vitro					
Reverse mutation (liquid fluctuation test, microtitre version)[a]	*S. typhimurium* (TA98, TA mix: TA7001 to TA7006)	*With S9*: 5.0 µg/mL 2-aminoanthracene for TA98 and TA mix *Without S9*: 0.25 µg/mL 2-nitrofluorene and 0.062 5 µg/mL 4-nitroquinoline-*N*-oxide for TA98 and TA mix	99.3% / LJ30551/65	Negative	Engelhardt & Leibold (2002)

[a] Not compliant with good laboratory practice; no test guideline.

Table 4. Summary of genotoxicity studies with metaflumizone metabolite M320I06

End-point	Test object	Concentrations	Purity/lot no.	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* (TA98, TA100, TA1535, TA1537)	12–5 000 µg/plate with/without S9	99.8%/L71-2	Negative	Schulz & Landsiedel (2009b)
	E. coli (WP2uvrA)	12–5 000 µg/plate with/without S9		Negative	
Gene mutation test (*Hprt* locus)	Chinese hamster ovary cells	125–1 500 µg/plate with/without S9	99.8%/L71-2	Negative	Schulz & Landsiedel (2009c)
Chromosomal aberration	Chinese hamster V79 cells	367.8–1 471.0 µg/plate with/without S9	99.8%/L71-2	Negative	Bohnenberger (2012)
In vivo					
Micronucleus test	In bone marrow cells of male NMRI mice	500, 1 000 and 2 000 mg/kg bw once by gavage	99.8%/L71-2	Negative	Schulz & Landsiedel (2009d)

bw: body weight; *Hprt*: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate

3. Observations in humans

One article was retrieved from the open literature that was considered to be relevant to this evaluation. A 57-year-old man presented with a Glasgow Coma Score of 4 following ingestion of a formulation containing 100 mL of metaflumizone, 150 mL of glyphosate and alcohol. Although the initial methaemoglobin level was slightly higher than the normal limit, it gradually rose to reach a maximum level of 27.8% 19 hours after ingestion. After haemodialysis, the methaemoglobin level was reduced to 15.8%, and it decreased further to 6% following methylene blue administration (Oh & Choi, 2014).

Metaflumizone shares a similar chemical structure with indoxacarb, which is a voltage-dependent sodium channel blocker insecticide and is known to cause methaemoglobinaemia. Oh & Choi (2014) suggested that physicians should be alert for the development of methaemoglobinaemia in symptomatic patients when facing potential metaflumizone poisoning.

The Meeting noted that the patient described in the above case-report was exposed to a mixture of chemicals and that there is no evidence of induction of methaemoglobinaemia in experimental animals exposed to metaflumizone.

Comments

Toxicological data

Further investigation of the brown pigment in the tubular epithelium of the kidney seen in the previously evaluated 1-year dog study confirmed that it was cytoplasmic lipofuscin (Kaspers, 2008).

Metaflumizone was negative in an Ames test (Schulz & Landsiedel, 2009a).

The Meeting concluded that the additional information provided on absent subclavian artery (Melching-Kollmuss, 2015; Schneider, 2015; DeSesso & Williams, 2017) in the previously evaluated developmental toxicity study in rabbits (Annex 1, reference *118*) supported the conclusion of the 2009 Meeting that metaflumizone is not teratogenic.

In an immunotoxicity study, female rats were administered metaflumizone by gavage at a dose of 0, 15, 40 or 75 mg/kg bw per day for 28 days. The NOAEL for systemic toxicity was 40 mg/kg bw per day, based on decreased body weight gain and feed consumption at 75 mg/kg bw per day. The NOAEL for immunotoxicity was 75 mg/kg bw per day, the highest dose tested (Buesen et al., 2011).

In a second immunotoxicity study, female rats were administered metaflumizone by gavage at 0, 15, 40 or 75 mg/kg bw per day for 28 days. The NOAEL for systemic toxicity was 40 mg/kg bw per day, based on reduced body weights and lower feed consumption at 75 mg/kg bw per day. The NOAEL for immunotoxicity was 75 mg/kg bw per day, the highest dose tested (Crittenden, 2011).

The Meeting concluded that metaflumizone is not immunotoxic.

The androgenic/anti-androgenic and estrogenic/anti-estrogenic activities of metaflumizone were investigated using two yeast screening assays. Metaflumizone showed no androgenic/anti-androgenic or estrogenic/anti-estrogenic activity (Woitkowiak, 2011a,b).

Toxicological data on metabolites

M320I04 (4-{2-oxo-2-[3-(trifluoromethyl)phenyl]ethyl}benzonitrile), a plant metabolite, has low acute toxicity (LD_{50} > 2000 mg/kg bw) (Gamer, 2002) and was not irritating to the skin or eyes of rabbits (Remmele, 2002a,b). It was negative for genotoxicity in a reverse mutation assay (Engelhardt & Leibold, 2002).

M320I06 (4-cyanobenzoic acid), a soil, plant and livestock metabolite, was not genotoxic in an adequate range of in vitro (Schulz & Landsiedel, 2009b,c; Bohnenberger, 2012) and in vivo studies (Schulz & Landsiedel, 2009d).

The Meeting concluded that M320I06 is not genotoxic.

Human data

A case-report of a poisoning incident did not provide any conclusive evidence regarding the toxicity of metaflumizone to humans (Oh & Choi, 2014).

Toxicological evaluation

The Meeting concluded that it was not necessary to revise the ADI or establish an ARfD for metaflumizone.

The Meeting concluded that plant and soil metabolite M320I23, evaluated by the 2009 Meeting, was of similar or lower toxicity relative to the parent compound and of similar chemical structure and therefore would be covered by the ADI for metaflumizone.

The Meeting concluded that metabolites M320I04 and M320I06, evaluated by the present Meeting, and soil metabolite M320I29, evaluated by the 2009 Meeting, were unlikely to be genotoxic. Following the "Plant and animal metabolite assessment scheme" of JMPR, the Meeting concluded that for chronic toxicity, these three metabolites could be assessed using the threshold of toxicological concern (TTC) approach. All three metabolites are categorized in Cramer class III, and therefore a TTC of 1.5 µg/kg bw per day applies.

The ADI applies to metaflumizone and M320I23, expressed as metaflumizone.

Acceptable daily intake (ADI) (applies to metaflumizone and M320I23, expressed as metaflumizone)

0–0.1 mg/kg bw

Critical end-points for setting guidance values for exposure to metaflumizone metabolites

Studies on metabolites	
M320I04 (plant metabolite)	Oral LD_{50} > 2 000 mg/kg bw
	No dermal irritation
	No ocular irritation
	No evidence of genotoxicity in vitro
M320I06 (soil, plant and livestock metabolite)	No evidence of genotoxicity

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THIABENDAZOLE (addendum)

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Explanation

Thiabendazole (2-(4-thiazolyl)-1*H*-benzimidazole) was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2006, when an acceptable daily intake (ADI) of 0–0.1 mg/kg body weight (bw) was established. The 2006 Meeting also established an acute reference dose (ARfD) of 1 mg/kg bw for the general population and an ARfD of 0.3 mg/kg bw for women of childbearing age (Annex 1, reference *109*).

Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, thiabendazole was placed on the agenda of the present Meeting, which assessed additional toxicological information available since the last review.

Several toxicological studies on thiabendazole were submitted to the present Meeting, including an acute neurotoxicity study, a 90-day neurotoxicity study and an immunotoxicity study.

All critical studies contained statements of compliance with good laboratory practice and were conducted in accordance with relevant national or international test guidelines, unless otherwise specified. One additional study that complemented the toxicological information submitted for the current assessment was identified from a literature search and was included in the evaluation.

Evaluation for acceptable intake

1. Toxicological studies

1.1 Long-term studies of toxicity and carcinogenicity

A carcinogenicity study, obtained from the open literature, was conducted using ICR mice (50 of each sex per group) treated with thiabendazole in the diet at a concentration of 0, 310, 1250 or 5000 parts per million (ppm) (equal to 0, 33.2, 146 and 605 mg/kg bw per day for males and 0, 40.0, 179 and 615 mg/kg bw per day for females, respectively) for 78 weeks.

There was no treatment-related effect on mortality in either sex. Body weights were depressed at 1250 ppm and above. The depression at 5000 ppm was more severe, approximately 20% and 15% for males and females, respectively. No treatment-related effects on haematology were observed. Major

treatment-related changes were found in the kidney at 1250 ppm and above and in the urinary bladder at 5000 ppm. At 1250 ppm, the incidence of nephrosis was increased in both sexes. At 5000 ppm, the incidences of calculi in the kidney and diffuse hyperplasia of urinary epithelium in the renal papilla and urinary bladder were increased. No treatment-related increase in tumours in any tissue was observed.

The no-observed-adverse-effect level (NOAEL) for long-term toxicity in mice was 310 ppm (equal to 33.2 mg/kg bw per day), based on body weight suppression and an increased incidence of nephrosis at 1250 ppm (equal to 146 mg/kg bw per day). No carcinogenicity was observed (Tada et al., 2001).

1.2 Special studies

(a) Acute neurotoxicity

An acute neurotoxicity study was conducted in non-fasted Crl:CD(SD) rats (10 of each sex per group) treated with thiabendazole (purity 99.3%; lot/batch no. H1998/011) in an aqueous 0.5% carboxymethylcellulose vehicle as a single gavage dose of 0, 50, 200 or 2000 mg/kg bw. The dosing volume was 5 mL/kg bw for all groups. Functional observational battery (FOB) assessments and locomotor activity (LMA) data were recorded for all animals prior to the initiation of dose administration, at time of peak effect (approximately 3 hours following dose administration) on study day 0, and on study days 7 and 14. On study day 15, rats were euthanized and necropsied.

All animals survived. There were no test substance–related clinical findings during the daily observations. Treatment-related changes in body weight were observed at 200 mg/kg bw and above in both sexes (Table 1). Body weights and body weight gains within 4 days after the treatment were statistically significantly decreased in both sexes at 2000 mg/kg bw, and body weight gains 1 day after the treatment were statistically significantly decreased in both sexes at 200 mg/kg bw. At 50 mg/kg bw, body weight gain 1 day after the treatment was slightly (approximately 2.5%), but also statistically significantly, decreased in females only. Statistically significant decreases in body weight or body weight gain within 4 days after the treatment were not observed in either sex at 50 mg/kg bw. This transient and slight decrease in body weight gain, which was not associated with other adverse effects in females at 50 mg/kg bw, was not considered to be an adverse effect of treatment. At 2000 and 200 mg/kg bw, feed consumption was decreased on the day of dosing (Table 1); subsequently, feed consumption was reduced at 2000 mg/kg bw only.

Treatment-related FOB findings were limited to the 200 and 2000 mg/kg bw males and females at time of peak effect (approximately 3 hours post-dosing) and included slight lacrimation and slightly drooping eyelids (2000 mg/kg bw females), decreased mean rearing counts (2000 mg/kg bw males and 200 and 2000 mg/kg bw females) and increased mean time to first step (2000 mg/kg bw females). The FOB findings were transient, as no test substance–related findings for these parameters were observed on study days 7 and 14. There were no FOB findings at 50 mg/kg bw.

No treatment-related findings were observed in sensory, neuromuscular or physiological observations or histopathological examinations.

LMA counts were varied, but statistically significant changes were observed on day 1 only (Table 1). The cumulative total LMA counts decreased in both sexes at 200 mg/kg bw and above, and the cumulative ambulatory LMA counts also decreased in males at 50 mg/kg bw and above and in females at 200 mg/kg bw and above. The decreases in ambulatory LMA counts were limited to within the first 20 minutes at 200 mg/kg bw and above, and no statistically significant decrease in ambulatory LMA count was found after 20 minutes except in the 2000 mg/kg bw group. The cumulative total LMA counts were comparable after day 1. The decreases observed at 200 mg/kg bw in both sexes within the first 20 minutes were slight but considered treatment related as a result of other treatment-related changes found at these doses.

A decrease in ambulatory LMA counts was noted in females at 50 mg/kg bw within 10 minutes, but no difference was found in subsequent subintervals on day 1 or the cumulative counts on day 1. The

decrease in ambulatory LMA counts in males at 50 mg/kg bw was not observed during the first 10 minutes and was not consistent with the decreases found at 200 mg/kg bw and above.

Table 1. Summary of acute neurotoxicity study in rats treated with thiabendazole

	Males				Females			
Parameter	**0 mg/kg bw**	**50 mg/kg bw**	**200 mg/kg bw**	**2 000 mg/kg bw**	**0 mg/kg bw**	**50 mg/kg bw**	**200 mg/kg bw**	**2 000 mg/kg bw**
Body weight gain (g) during days 0–1[a]	5 ± 2.3	1 ± 2.9	−7 ± 5.3**	−13 ± 6.8**	3 ± 3.3	−1 ± 2.5*	−6 ± 3.3**	−8 ± 3.6**
Body weight gain (g) during days 1–2[a]	8 ± 3.3	9 ± 3.5	12 ± 3.5**	−5 ± 3.6**	5 ± 2.6	4 ± 5.3	6 ± 2.3	−6 ± 3.5*
Body weight gain (g) during days 7–8[a]	7 ± 1.4	6 ± 3.7	6 ± 4.5	7 ± 2.3	2 ± 5.6	2 ± 4.1	2 ± 5.2	2 ± 3.6
Feed consumption on day of dosing (g/rat per day)	21	19	13**	8**	16	14	9**	6**
Total locomotor activity counts[a]								
Pretest (cumulative[b])	2 010 ± 478	2 542 ± 581	2 341 ± 396	1 956 ± 536	2 237 ± 434	2 180 ± 443	1 846 ± 637	2 043 ± 600
Day 1[c] (cumulative[b])	2 178 ± 761	1 705 ± 667	1 333 ± 387**	1 069 ± 455**	2 524 ± 622	2 362 ± 776	1 586 ± 530**	926 ± 477**
Day 7 (cumulative[b])	2 428 ± 107	2 616 ± 533	2 519 ± 467	1 852 ± 767	2 710 ± 1 125	3 223 ± 1 121	2 963 ± 950	3 016 ± 758
Ambulatory locomotor activity counts[a]								
Pretest (cumulative[b])	475 ± 181	560 ± 212	593 ± 234	479 ± 198	584 ± 199	575 ± 157	466 ± 188	519 ± 239
Day 1[c] (cumulative[b])	550 ± 232	388 ± 150*	299 ± 125**	241 ± 124**	730 ± 185	609 ± 205	394 ± 143**	238 ± 137**
Subinterval on day 1[c]								
0–10 minutes	375 ± 111	305 ± 105	222 ± 87**	136 ± 101**	565 ± 121	417 ± 103**	305 ± 106**	121 ± 82**
11–20 minutes	128 ± 114	62 ± 53*	24 ± 31**	22 ± 26**	102 ± 106	105 ± 79	35 ± 57**	17 ± 28*
21–30 minutes	25 ± 37	8 ± 18	19 ± 29	24 ± 22	42 ± 62	72 ± 90	32 ± 68	12 ± 18
31–40 minutes	7 ± 14	5 ± 9	16 ± 23	25 ± 24	12 ± 32	9 ± 21	10 ± 19	45 ± 71
41–50 minutes	0 ± 1	8 ± 22	14 ± 23	14 ± 15	1 ± 4	5 ± 14	9 ± 28	18 ± 19*
51–60 minutes	14 ± 42	1 ± 2	5 ± 9	20 ± 15	8 ± 16	0 ± 0.4	3 ± 8	24 ± 23*
Day 7 (cumulative[b])	511 ± 238	593 ± 186	568 ± 224	353 ± 176	793 ± 503	814 ± 308	811 ± 322	759 ± 210

bw: body weight; *: $P \leq 0.05$; **: $P \leq 0.01$

[a] All changes are expressed as mean ± standard deviation.

[b] Cumulative during 0–60 minutes.

[c] Three hours after dosing.

Source: Herberth (2012a)

The decreases in the ambulatory LMA counts at 50 mg/kg bw on study day 0 were not considered adverse for the following reasons:

1) The results seen at 50 mg/kg bw were within the range of the concurrent control values, which showed the high level of variability typically seen in these measurements.
2) The decrease observed at 50 mg/kg bw was transient at subintervals of 11–20 minutes in males and 0–10 minutes in females on day 0. This transient decrease did not result in a statistically significant decrease in the cumulative LMA counts in females at 50 mg/kg bw on day 1.
3) No treatment-related neurological effects were observed at 50 mg/kg bw.

The NOAEL for systemic toxicity was 50 mg/kg bw, based on decreases in mean rearing counts in females, body weight loss secondary to reduced feed consumption, and lower ambulatory LMA counts at the time of peak effect on study day 0 in both sexes at 200 mg/kg bw. There was no clear evidence that thiabendazole was acutely neurotoxic (Herberth, 2012a).

(b) Short-term studies of neurotoxicity

In a 90-day neurotoxicity study, thiabendazole (purity 99.3%; lot/batch no. HK1998/011) was administered to Crl:CD(SD) rats (12 of each sex per group) in the diet at 0, 200, 750 or 1500 ppm (equal to 0, 13, 47 and 95 mg/kg bw per day for males and 0, 15, 54 and 108 mg/kg bw per day for females, respectively). FOB and LMA data were recorded for all animals during the pretest and then during the second, fourth, eighth and thirteenth weeks of test diet administration (study weeks 1, 3, 7 and 12, respectively). Ophthalmic examinations were performed. Five rats of each sex per group were deeply anaesthetized and perfused in situ during study week 13; brain weights and brain dimensions (excluding olfactory bulbs) were recorded. Neuropathological examination of the rats in the 0 and 1500 ppm groups was performed after perfusion fixation.

All animals survived to the scheduled euthanasia. There were no test substance–related clinical findings during the weekly examinations.

Treatment-related lower mean body weight gains, with corresponding decrements in feed consumption, were generally noted for the 1500 ppm males and females throughout the exposure period. These changes were occasionally statistically significant, resulting in a statistically significantly lower overall (study days 0–91) mean body weight gain for both males (11.3% on day 91) and females (17.4% on day 91) at 1500 ppm. As a result, mean absolute body weights for males and females were lower than the control values beginning on study day 7 and continuing throughout the study; the differences were statistically significant during study days 84–91 for males (18.3%) and study days 14–91 for females (36.1%). Feed consumption was lower in males (8%) and females (17%) at 1500 ppm throughout the study.

No treatment-related changes were observed in clinical signs, FOB evaluations, mean total and ambulatory LMA counts, the pattern of habituation, ophthalmology, neuropathology, and weights and measurements of the brain in either sex in any treatment group.

The NOAEL for systemic toxicity was 750 ppm (equal to 47 mg/kg bw per day), based on decreased body weight gains, lower body weights and decreased feed consumption at 1500 ppm (equal to 95 mg/kg bw per day).

The NOAEL for neurotoxicity was 1500 ppm (equal to 95 mg/kg bw per day), the highest dose tested (Herberth, 2012b).

(c) Immunotoxicity

In an immunotoxicity study, thiabendazole (purity 99.3%; batch no. HK1998/011) was administered to female CD-1 mice (10 per group) in the diet at a concentration of 0, 100, 1000 or 5000 ppm (equal to 0, 20.9, 205.6 and 1027.0 mg/kg bw per day, respectively) for 28 days. The concurrent vehicle control group and the positive control group were offered the basal (untreated) diet on a comparable regimen to the thiabendazole-treated groups. All mice received an intravenous injection of sheep red blood cells on study day 24. Mice in the positive control group were administered the positive control substance, cyclophosphamide, via intraperitoneal injection (50 mg/kg bw per day) once daily for 4 days (study days 24 through 27). All animals were euthanized on study day 28. Histopathological examination was not conducted.

Lower body weights were observed in the 5000 ppm group from study days 0 to 3, but the values were generally similar to those of the vehicle control group for the remainder of the study. Thiabendazole-related increases in absolute liver weights were noted in the 1000 and 5000 ppm groups; however, when adjusted for terminal body weight, statistically significantly higher (41.2%) liver weight was noted only at 5000 ppm.

In the functional immune evaluation of the immunoglobulin M (IgM) antibody-forming cell (AFC) response, there was a thiabendazole-related lower total spleen activity, measured as IgM antibody-forming cells per spleen (AFC/spleen), at 5000 ppm (53%) when compared with the vehicle control group; however, thiabendazole did not significantly suppress spleen cell numbers or specific IgM antibody-forming cell activity (AFC/10^6 spleen cells) at any dose.

For the positive control (cyclophosphamide) group, statistically significantly lower spleen and thymus weights, spleen cell numbers, specific activity and total spleen activity of IgM antibody-forming cells were noted when compared with the vehicle control group; these effects were consistent with the known immunosuppressant effects of cyclophosphamide and validated the functionality of the assay.

The NOAEL for immunotoxicity was 1000 ppm (equal to 205.6 mg/kg bw per day), based on lower total spleen activity measured as IgM antibody-forming cells per spleen at 5000 ppm (equal to 1027.0 mg/kg bw per day).

The NOAEL for systemic toxicity was 1000 ppm (equal to 205.6 mg/kg bw per day), based on lower body weights and markedly increased liver weights at 5000 ppm (equal to 1027.0 mg/kg bw per day) (Wasil, 2012).

Comments

Toxicological data

In a chronic toxicity and carcinogenicity study, mice were administered thiabendazole in the diet at 0, 310, 1250 or 5000 ppm (equal to 0, 33.2, 146 and 605 mg/kg bw per day for males and 0, 40.0, 179 and 615 mg/kg bw per day for females, respectively) for 78 weeks. The NOAEL for long-term toxicity in mice was 310 ppm (equal to 33.2 mg/kg bw per day), based on body weight suppression and an increased incidence of nephrosis at 1250 ppm (equal to 146 mg/kg bw per day). No carcinogenicity was observed (Tada et al., 2001).

In an acute neurotoxicity study in rats treated with thiabendazole as a single dose of 0, 50, 200 or 2000 mg/kg bw by gavage, the NOAEL for systemic toxicity was 50 mg/kg bw, based on decreases in mean rearing counts in females, body weight loss secondary to reduced feed consumption, and lower ambulatory LMA counts at the time of peak effect on study day 0 in both sexes at 200 mg/kg bw. There was no clear evidence that thiabendazole was acutely neurotoxic (Herberth, 2012a).

In a 90-day neurotoxicity study in rats treated with thiabendazole in the diet at 0, 200, 750 or 1500 ppm (equal to 0, 13, 47 and 95 mg/kg bw per day for males and 0, 15, 54 and 108 mg/kg bw per day for females, respectively), the NOAEL for systemic toxicity was 750 ppm (equal to 47 mg/kg bw per day), based on findings of decreased body weight gain, depressed body weights and lower feed consumption at 1500 ppm (equal to 95 mg/kg bw per day). The NOAEL for neurotoxicity was 1500 ppm (equal to 95 mg/kg bw per day), the highest dose tested (Herberth, 2012b).

The Meeting concluded that thiabendazole is not neurotoxic.

In an immunotoxicity study in female mice treated with thiabendazole in the diet at a concentration of 0, 100, 1000 or 5000 ppm (equal to 0, 20.9, 205.6 and 1027.0 mg/kg bw per day, respectively) for 28 days, the NOAEL for immunotoxicity was 1000 ppm (equal to 205.6 mg/kg bw per day), on the basis of lower total spleen activity measured as IgM antibody-forming cells per spleen at 5000 ppm (equal to 1027.0 mg/kg bw per day). The NOAEL for systemic toxicity was 1000 ppm (equal to 205.6 mg/kg bw per day), based on reduced body weight and marked increases in liver weights at 5000 ppm (equal to 1027.0 mg/kg bw per day) (Wasil, 2012).

The Meeting concluded that thiabendazole is not immunotoxic in the absence of systemic toxicity.

Toxicological evaluation

The Meeting concluded that no revision of the ADI or ARfDs was necessary. The Meeting noted that the NOAEL for systemic toxicity from the acute neurotoxicity study (50 mg/kg bw) is lower than the NOAEL from the study currently used in the derivation of the ARfD for the general population (100 mg/kg bw). However, as the lowest-observed-adverse-effect level (LOAEL) for both studies is 200 mg/kg bw, and as the findings in both studies are similar, the Meeting concluded that there was no reason to revise the ARfD for the general population.

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

Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body weight suppression, nephrosis
Lowest relevant NOAEL	33.2 mg/kg bw per day (mouse)
Carcinogenicity	No evidence of carcinogenicity
Neurotoxicity	
Acute neurotoxicity NOAEL	2000 mg/kg bw, highest dose tested (rat) Systemic toxicity NOAEL 50 mg/kg bw (rat)
Ninety-day neurotoxicity NOAEL	95 mg/kg bw per day, highest dose tested (rat)
Other toxicological studies	
Immunotoxicity NOAEL	205.6 mg/kg bw per day (mouse); not immunotoxic in the absence of systemic toxicity

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ANNEX 1

Reports and other documents resulting from previous Joint Meetings of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues

1. Principles governing consumer safety in relation to pesticide residues. Report of a meeting of a WHO Expert Committee on Pesticide Residues held jointly with the FAO Panel of Experts on the Use of Pesticides in Agriculture. FAO Plant Production and Protection Division Report, No. PL/1961/11; WHO Technical Report Series, No. 240, 1962.
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3. Evaluation of the toxicity of pesticide residues in food. Report of the Second Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues. FAO Meeting Report, No. PL/1965/10; WHO/Food Add./26.65, 1965.
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This volume contains toxicological monograph addenda that were prepared by the 2019 Extra Joint FAO/WHO Meeting on Pesticide Residues (JMPR), which met in Gatineau, Canada, on 7–17 May 2019.

The monograph addenda in this volume summarize the newly submitted toxicological data on eight pesticides that could leave residues in food commodities. These pesticides are acetochlor, boscalid, chlorothalonil, cyprodinil, dicamba, mesotrione, metaflumizone and thiabendazole. The data summarized in the toxicological monograph addenda served as the basis for reconsidering the acceptable daily intakes and acute reference doses that were previously established by the Meeting and for evaluating the toxicity of metabolites and/or degradates of the pesticides under consideration.

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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