AMETOCTRADIN

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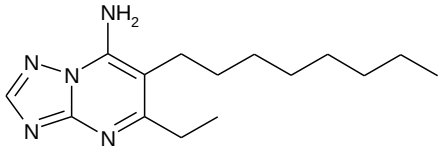
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Explanation

Ametoctradin (Figure 1) is the common name provisionally approved by the International Organization for Standardization (ISO) for 5-ethyl-6-octyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine, for which the Chemical Abstracts Service number is 865318-97-4. Ametoctradin is a fungicide that inhibits zoospore differentiation within the zoosporangium, the release of zoospores from the zoosporangium, the motility of any released zoospores and the germination of encysted zoospores. It acts by reducing the adenosine triphosphate content in these stages of development by binding to and inhibiting complex III of the respiratory chain in mitochondria of oomycetes.

Ametoctradin has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues.

Figure 1. Structure of ametoctradin



All studies evaluated in this monograph were performed by good laboratory practice–certified laboratories and complied with the relevant Organisation for Economic Co-operation and Development and/or United States Environmental Protection Agency test guidelines.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The absorption, distribution, metabolism and elimination of ^{14}C-labelled ametoctradin were investigated in male and female Wistar rats at doses of 20, 100, 500 and 1000 mg/kg body weight (bw) for plasma kinetics and 50 and 500 mg/kg bw for mass balance, tissue distribution and biliary excretion experiments. The experiments were performed with ametoctradin labelled in the pyrimidine and triazole rings. In order to achieve the required specific activity, the respective amounts of non-labelled material were added to the radiolabelled material. To facilitate elucidation of the structures of the metabolites formed, ^{13}C-labelled material was mixed with unlabelled ametoctradin at a ratio of 1:1 and added to the respective amounts of ^{14}C-labelled ametoctradin. The study design is summarized in Table 1.

The dermal absorption of ametoctradin in the formulation BAS 651 00 F was investigated in vitro in human and rat skin.

Blood/plasma levels study: ^{14}C-labelled ametoctradin was administered to groups of four male and four female Wistar rats in a single oral dose of 20, 100, 500 or 1000 mg/kg bw. Blood samples were collected at 1, 2, 4, 8, 24, 48, 72 and 96 hours after administration.

Following a single oral dose of ^{14}C-labelled ametoctradin, maximum plasma concentrations were observed 1 hour after dosing, except in females dosed with 500 mg/kg bw, for which maximum plasma concentrations were observed 2 hours post-dosing. Afterwards, plasma levels declined until sacrifice at 96 hours in all dose groups to values of about 0.01–0.2 µg equivalent (Eq) per gram plasma. Initial half-lives in plasma ranged from 2.13 to 2.91 hours for males and from 1.74 to 2.51 hours for females (Table 2). The area under the plasma concentration–time curve (AUC) values indicate a sex-independent internal exposure and show that there is not a linear correlation between the internal exposure (measured as total radioactivity) and increasing external dose.

Mass balance/excretion study: Groups of Wistar rats (four of each sex) received radiolabelled ametoctradin as a single dose of 50 mg/kg bw, a single dose of 500 mg/kg bw or a single dose of 500 mg/kg bw following by 14 repeated daily doses of unlabelled compound at 500 mg/kg bw. Urine was collected after 6, 12 and 24 hours, then at intervals of 24 hours up to 168 hours, and faeces were collected at intervals of 24 hours up to 168 hours. After 168 hours, animals were sacrificed, and tissues were collected.

Total recovery of radioactivity at 168 hours ranged from 91% to 110% of the administered dose. The main route of elimination was via faeces for all exposure regimens (from 73% to 102%; Table 3). Faecal elimination was nearly complete by 48 hours, with 71.9–82.1% for the low-dose group, 98.4–99.0% for the single high dose group and 82.4–94.2% for the repeated high dose group. Urinary excretion accounted for 4.8–21.8% and was also complete within 48 hours after

administration. After 168 hours, only small amounts of radioactivity were present in tissues and carcass.

Table 1. Study design for biokinetic studies with ametoctradin

Test group	Dose (mg/kg bw)	No. of rats	Study design
Blood/plasma levels study			
Experiment 1	1000 (^{14}C)	4 M, 4 F	Retro-orbital blood sampling was conducted at 1, 2, 4, 8, 24, 48 and 72 h; exsanguinations were performed at 96 h.
Experiment 2	500 (^{14}C)	4 M, 4 F	
Experiment 3	100 (^{14}C)	4 M, 4 F	
Experiment 4	20 (^{14}C)	4 M, 4 F	
Mass balance/excretion study			
Experiment 5	500 (^{14}C)	4 M, 4 F	Excreta and expired air were determined by means of metabolism cages. Urine was collected after 6, 12 and 24 h and subsequently at 24 h time intervals. Exhaled air was collected for 48 h. Animals were sacrificed and tissues were collected at 168 h.
Experiment 6	50 (^{14}C and ^{13}C)	4 M, 4 F	
Experiment 7	500 (14× unlabelled followed by ^{14}C-labelled)	4 M, 4 F	
Tissue distribution study			
Experiment 8	500 (^{14}C)	12 M, 12 F	Sacrifice times, 500 mg/kg bw:
Experiment 9	50 (^{14}C)	12 M, 12 F	M: 1, 4, 16, 22 h
			F: 1, 2, 8, 20 h
			Sacrifice times, 50 mg/kg bw:
			M/F: 1, 2.5, 8, 20 h (3 of each sex per time point)
			Tissue analysis, total radioactivity
Biliary excretion study			
Experiment 10	500 (^{14}C and ^{13}C)	4 M, 4 F	Bile was collected at 3 h intervals for up to 72 h. In addition, urine and faeces were collected at 24 h time intervals up to 72 h. After sacrifice, stomach, gut and carcass were checked for remaining radioactivity. In addition, cage wash was checked for radioactivity.
Experiment 11	50 (^{14}C and ^{13}C)	4 M, 4 F	

From Fabian & Landsiedel (2008)
F, female; M, male

Table 2. Pharmacokinetic parameters of ^{14}C-labelled ametoctradin administered to rats

Sex	Dose (mg/kg bw)	C_{max} (µg Eq/g)	T_{max} (h)	Half-life initial (h)	Half-life intermediate (h)	Half-life terminal (h)	AUC (µg Eq·h/g)
Male	20	0.83	1	2.13	7.71	20.67	6.5
	100	2.43	1	2.42	7.51	31.33	23.0
	500	6.54	1	2.91	8.71	31.21	66.9
	1000	12.53	1	2.54	10.18	29.12	136.4
Female	20	0.73	1	2.51	—	20.67	5.0
	100	2.86	1	1.74	9.21	29.13	22.0
	500	10.32	1	n.r.[a]	8.58	29.88	79.9
	1000	13.10	1	1.91	11.03	28.53	126.1

From Fabian & Landsiedel (2008)
AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; n.r., not reliable; T_{max}, time to reach C_{max}

[a] The value of 1.18 given by the study author is considered not reliable, as the plasma value at 1 hour after dosing is not a maximum; the plasma value at 2 hours after dosing is the actual maximum.

Table 3. Recovery of radioactivity in rats up to 168 hours after oral exposure to ametoctradin

Sample	Time interval (h)	Recovery of radioactivity (% of administered dose)					
		50 mg/kg bw single dose		500 mg/kg bw single dose		(14× + 1) × 500 mg/kg bw per day repeated dose	
		M	F	M	F	M	F
Faeces	0–24	57.48	62.21	91.40	76.00	76.44	64.97
	24–48	14.42	19.91	7.02	23.02	17.72	17.46
	48–72	1.04	1.39	1.39	1.03	1.29	0.93
	72–96	0.21	0.27	0.18	0.17	1.05	0.13
	96–120	0.04	0.11	1.84	0.08	0.05	0.96
	120–144	0.03	0.12	0.06	0.06	1.69	0.06
	144–168	0.23	0.05	0.04	0.05	0.04	0.06
	0–168[a]	73.44	84.05	101.91	100.41	98.28	84.56
Urine	0–6	9.82	8.87	3.24	4.05	1.99	2.01
	6–12	4.23	4.95	0.93	0.96	0.88	0.20
	12–24	3.30	4.57	1.16	0.99	0.84	1.83
	24–48	1.66	2.31	0.88	2.01	0.76	1.57
	48–72	0.21	0.56	0.16	0.31	0.15	0.19
	72–96	0.06	0.22	0.09	0.10	0.06	0.12
	96–120	0.05	0.14	0.05	0.04	0.03	0.05
	120–144	0.04	0.11	0.03	0.03	0.02	0.04
	144–168	0.02	0.09	0.01	0.02	0.02	0.04
	0–168[a]	19.37	21.81	6.51	8.51	4.76	6.03
Cage wash	—	0.09	0.35	0.16	0.10	0.14	0.36
Tissue[b]	—	0.12	0.07	0.53	0.43	0.29	0.28
Carcass	—	0.06	0.08	0.31	0.29	0.23	0.27
Total recovery	0–168	93.18	106.36	109.40	109.73	103.72	91.49

From Fabian & Landsiedel (2008)
F, female; M, male
[a] Numbers may not add exactly due to rounding.
[b] Tissues including skin and blood.

Tissue distribution study: Groups of 12 male and 12 female rats were administered a single oral dose of 50 or 500 mg/kg bw. Three animals of each sex were killed at four different time points based on the determined maximum plasma concentrations—namely, C_{max}, ½ C_{max}, ¼ C_{max} and ⅛ C_{max} (see Table 1).

After a single oral dose, ametoctradin was widely distributed, with the highest mean tissue concentrations of radioactivity for the low-dose group of males occurring after 1–2.5 hours in the stomach and stomach contents (174.8–801.0 µg Eq/g) and gut and gut contents (78.9–706.9 µg Eq/g). For the non-gastrointestinal tract tissues, the highest levels were found in the liver (33.4 µg Eq/g) and kidneys (15.2 µg Eq/g). In low-dose females, the highest mean tissue concentrations were found after 1–2.5 hours in the same tissues at similar concentrations. For the males in the high-dose group, the highest mean tissue concentrations were found after 1–4 hours, also in the stomach and stomach contents (1198.6–19 352.5 µg Eq/g), gut and gut contents (31.3–202.3 µg Eq/g), thyroid (64.3 µg

Eq/g), liver (31.3 µg Eq/g) and kidneys (22.4 µg Eq/g). In high-dose females, the highest mean tissue concentrations were found after 1–4 hours in the same tissues.

Biliary excretion study: Groups of bile duct–cannulated Wistar rats (four of each sex) received a single oral dose of 50 or 500 mg/kg bw. Bile was collected at 3-hour intervals and urine and faeces were collected at 24-hour intervals for up to 72 hours. The gastrointestinal tract was collected at termination.

Biliary excretion was lower in females than in males in both dose groups, with 12.4% and 22.5% of the administered dose recovered in low-dose females and low-dose males, respectively, and 3.2% and 10.9% in high-dose females and high-dose males, respectively. Biliary excretion took place mainly during the first 48 hours and gradually decreased thereafter. Based on the amount of radioactivity excreted via bile and urine and the radioactivity in cage wash and carcass, the bioavailability of radiolabelled ametoctradin was calculated to be about 36% and 42% in low-dose males and low-dose females, respectively, and about 23% and 16% in high-dose males and high-dose females, respectively (Fabian & Landsiedel, 2008).

Dermal absorption study: No dermal absorption study on the pure active substance is available. The dermal absorption of ametoctradin (purity 99.1%) was studied in vitro in human and rat skin membranes using BAS 651 00 F, a suspension concentrate formulation containing ametoctradin at 300 g/l and dimethomorph at 225 g/l. Ametoctradin was applied at concentrations of 2948 and 1.8 µg active substance per square metre under semi-occluded dressing for 24 hours. As increasing levels of radiolabel were found in receptor fluid from 1 to 24 hours and as ametoctradin is lipophilic, the amount of radiolabel retained in the skin and tape strips should be included to estimate the potential dermal absorption. After 24 hours, 1% and 5% of the applied dose were absorbed following application of the high and low concentrations to rat skin, respectively. For human skin, the corresponding percentages were 0.5% and 3% for the high and low doses, respectively (Gamer, Fabian & Landsiedel, 2007).

1.2 Metabolism

The metabolic fate of ^{14}C-labelled ametoctradin was investigated in a follow-up study. Urine, faeces and bile samples originating from the biokinetics study (Fabian & Landsiedel, 2008) were used to investigate metabolite patterns. Three additional groups of animals were dosed with ^{14}C-labelled ametoctradin specifically for the metabolism study. A summary of the study design is given in Table 4. In dose group DX, urine and faeces were collected at 24-hour intervals over 4 days for female rats and 7 days for male rats. In dose groups V and W, animals were sacrificed at 1 hour post-dosing, and plasma, liver and kidney samples were collected. Urine and bile, extracts of faeces, liver and kidney tissues and plasma were analysed by high-performance liquid chromatography.

Table 4. Study design for metabolism study with ^{14}C-labelled ametoctradin administered to rats

Test group	Dose of labelled material (mg/kg bw)	No. of each sex	Determination
Group B (Experiment 6, Table 1)	50 (^{14}C and ^{13}C)	4	See Table 1
Group C (Experiment 7, Table 1)	500 (14× unlabelled followed by ^{14}C-labelled)	4	
Group D (Experiment 5, Table 1)	500 (^{14}C)	4	
Group R (Experiment 11, Table 1)	50 (^{14}C and ^{13}C)	4	See Table 1
Group S (Experiment 10, Table 1)	500 (^{14}C and ^{13}C)	4	
Group DX	500 (male) / 250 (female) (^{14}C and ^{13}C)	10	Urine and faeces collected at 24 h intervals
Group V	50 (^{14}C and ^{13}C)	4	Liver, kidney and plasma

Test group	Dose of labelled material (mg/kg bw)	No. of each sex	Determination
Group W	500 (^{14}C and ^{13}C)	4	collected after 1 h (C_{max})

From Hafemann & Kloeppner (2008)

Three metabolites, which were generated by full oxidation of the octyl side-chain with subsequent degradation of C-2 or C-1 units, were identified in urine. The ω-hetarylhexanoic acid M650F06 was the most abundant metabolite (5–8% of the administered dose at the low dose and 2–8% at the high dose), followed by the ω-hetarylbutanoic acid M650F01 (0.5–1% at the low dose and 0.3–1% at the high dose) and the ω-hetarylpentanoic acid M650F05 (0.2% at the low dose and 0.05–0.2% at the high dose). In faeces, the excreted radioactivity consisted primarily of the unchanged parent compound (43–69% for the low-dose group, 65–92% for the high-dose group). As in urine, the main metabolites in faeces were M650F06 (11–20% at the low dose and 4–18% at the high dose) and M650F01 (2–5% at the low dose and 0–3% at the high dose). No clear sex-specific differences were observed in either urine or faeces. In bile, the major metabolite in both dose groups and sexes was M650F06 (8–13% at the low dose and 2–5% at the high dose). In addition, two metabolites derived from metabolite M650F06 by conjugation with taurine (M650F12; 1–4% at the low dose and < 0.1–2% at the high dose) or glucuronic acid (M650F11; 0.2–1% at the low dose and < 0.1–1% at the high dose) were identified. Moreover, non-conjugated metabolites were identified as M650F01 (1–3% at the low dose and 0.2–1% at the high dose) and the ω-hetaryloctanoic acid (M650F09; 0.7–0.8% at the low dose and 0–0.2% at the high dose). Another metabolite deriving from metabolite M650F09 by conjugation with taurine was identified to be M650F10. This metabolite was observed only in male rats (3% at the low dose and 2% at the high dose). Overall, the total quantity of metabolites in bile was greater in males than in females. In liver and plasma, M650F06, M650F09 and parent compound were identified. In kidney, the metabolite patterns were comparable to those in liver and plasma, although parent compound was not detected. The total amount of parent compound and metabolites found in liver, kidney and plasma was less than 1% of the administered dose. The proposed metabolic pathway of ametoctradin in the rat is shown in Figure 2 (Hafemann & Kloeppner, 2008).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The acute toxicity of ametoctradin is summarized in Table 5. Ametoctradin has low acute toxicity in rats via the oral, dermal and inhalation routes. No substance-related clinical signs were observed after oral or dermal administration. In the inhalation studies, clinical signs of toxicity included visually increased respiration, squatting posture, piloerection, and smeared and contaminated fur.

(b) Dermal irritation

In a dermal irritation study, three male New Zealand White rabbits were exposed to 0.5 g ametoctradin (purity 99.3%, batch COD-000748) applied to a 6.25 cm^2 area of intact skin of the flank under semi-occluded dressing for 4 hours. Skin reactions were scored at 1, 2, 24, 48 and 72 hours following patch removal. Slight erythema was observed in all animals immediately after removal of the patch and after 1 hour. In two out of three animals, the slight erythema was still visible after 24 hours. The cutaneous reactions were reversible in all animals within 48 hours after removal of the patch. No signs of oedema were observed during the study. The individual average scores (24–72 hours) for erythema of each rabbit were 0.3, 0.0 and 0.3, and for oedema, 0.0. It was concluded that ametoctradin does not show a skin irritation potential (Remmele & Landsiedel, 2007a).

In a second dermal irritation study, three male New Zealand White rabbits were cutaneously exposed to 0.5 g ametoctradin (purity 100%, batch F15101209/022) applied to a 6.25 cm^2 area of intact skin of the flank under semi-occluded dressing for 4 hours. Skin reactions were scored at 1, 2,

24, 48, 72 and 96 hours following patch removal. Slight erythema was observed in all animals immediately after removal of the patch. The skin reactions were reversible in all animals within 1 hour after patch removal. The individual average scores (24–72 hours) for erythema as well as oedema of each rabbit were in all cases 0.0. It was concluded that ametoctradin is not a skin irritant (Cords & Lammer, 2010d).

Figure 2. Proposed metabolic pathway of ametoctradin in rat

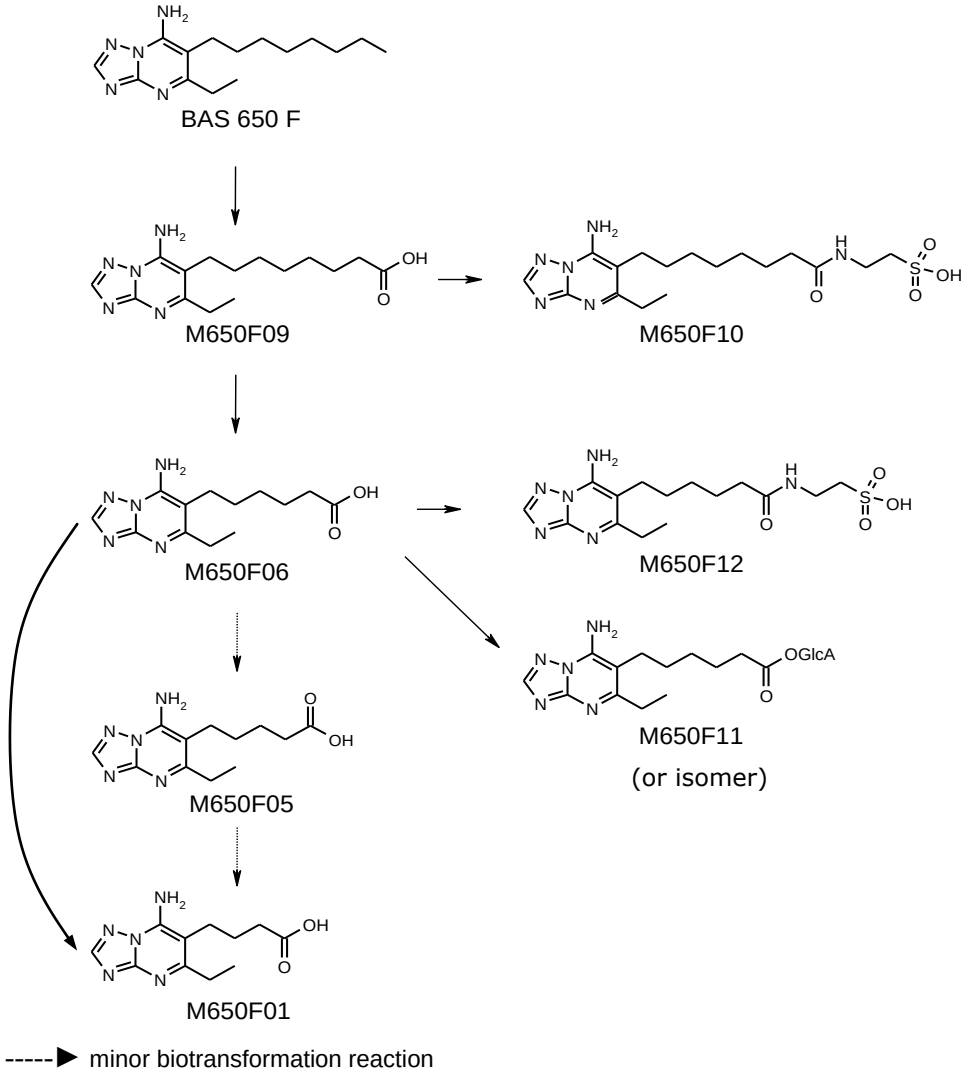


Table 5. Acute toxicity of ametoctradin

Species	Strain	Sex	Route	Purity; batch	LD_{50}	Reference
Rat	Wistar	Female	Oral	99.3%; COD-000748	> 2000 mg/kg bw	Gamer & Landsiedel (2007a)
Rat	Wistar	Female	Oral	100%; F15101209/022	> 2000 mg/kg bw	Cords (2010a); Cords & Lammer (2010a)
Rat	Wistar	Male and female	Oral	100%; F15101209/022	> 5000 mg/kg bw	Cords & Lammer (2010b)
Rat	Wistar	Male and female	Dermal	99.3%; COD-000748	> 2000 mg/kg bw	Gamer & Landsiedel (2007b)

Species	Strain	Sex	Route	Purity; batch	LD_{50}	Reference
Rat	Wistar	Male and female	Dermal	100%; F15101209/022	> 2000 mg/kg bw	Cords (2010b); Cords & Lammer (2010c)
Rat	Wistar	Male and female	Inhalation	99.3%; COD-000748	> 5.5 mg/l	Ma-Hock & Landsiedel (2006)
Rat	Wistar	Male and female	Inhalation	100%; F15101209/022	> 5.4 mg/l	Wittmer & Mellert (2010)

LD_{50}, median lethal dose

(c) Ocular irritation

In a study on the eye irritation potential of ametoctradin, 0.1 ml (equivalent to 34 mg) ametoctradin (purity 99.3%, batch COD-000748) was applied into the conjunctival sac of the right eye of three New Zealand White rabbits. The eyes were rinsed with tap water 1 hour after application. Ocular lesions were scored at 1, 24, 48 and 72 hours after application, and signs of irritation were scored according to Draize. No signs of systemic toxicity or mortality were observed during the study period. Slight or moderate conjunctival redness and slight discharge were observed in all three animals at the 1-hour observation period. The ocular reactions were reversible in two animals within 24 hours and in one animal within 48 hours after application. Mean scores for each animal at the 24-, 48- and 72-hour observations were 0.0 for corneal opacity, iris lesions and chemosis and 0.0, 0.0 and 0.3 for conjunctival redness. It was concluded that ametoctradin is non-irritating to eyes (Remmele & Landsiedel, 2007b).

Three adult female New Zealand White rabbits were exposed to 0.1 ml (about 34 mg) ametoctradin (purity 99.3%, batch COD-000748) applied into the conjunctival sac of the right eye. The eyes were rinsed with tap water 24 hours after application, and ocular lesions were scored at 1, 24, 48 and 72 hours. At 1 hour, ocular reactions comprised slight or moderate conjunctival redness, slight conjunctival chemosis and slight discharge. The ocular reactions were reversible in all animals within 24 hours after application. The average scores (24–72 hours) for each rabbit were 0.0 for corneal opacity, iris lesions, conjunctival redness and chemosis. It was concluded that ametoctradin was not an eye irritant (Remmele & Landsiedel, 2007c).

In an eye irritation study, 0.1 ml (about 33 mg) of ametoctradin (purity 100%, batch F15101209/022) was applied into the conjunctival sac of the right eye of three adult female New Zealand White rabbits. About 24 hours after application, the eyes were rinsed with tap water. The rabbits in this study were observed for 7 days following treatment. The ocular reactions were assessed at 1, 24, 48, 72 and 96 hours after application as well as on study day 7. Ocular reactions included slight conjunctival redness in all animals 1 hour after application and persisted in one out of three animals up to 48 hours, in the second animal up to 72 hours and in the third animal up to 96 hours. Slight conjunctival chemosis was noted in one animal 1 hour after application up to 24 hours. The ocular reactions were reversible in all animals within 7 days. Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0 for all animals for corneal opacity and iris lesions, 1.0, 1.0 and 0.7 for redness of the conjunctiva and 0.3, 0.0 and 0.0 for chemosis. It was concluded that ametoctradin is non-irritating to eyes (Cords & Lammer, 2010e).

(d) Dermal sensitization

The skin sensitization potential of ametoctradin (purity 99.3%, batch COD-000748) was investigated using the murine local lymph node assay. Groups of six female CBA/Ca mice were treated with 10%, 30% or 50% weight per weight (w/w) preparations. Higher concentrations were not technically achievable. The vehicle was propylene glycol. For 3 consecutive days, 25 µl of the respective test substance or vehicle control alone was applied to the dorsum of both ears. On day 5, the mice received 0.74 MBq of ^{3}H-labelled thymidine in 250 µl of sterile saline by intravenous injection into a tail vein. About 5 hours after ^{3}H-labelled thymidine treatment, the mice were sacrificed, and the auricular lymph nodes were removed. From each animal, ear thickness, ear weight

and lymph node weight were determined, in addition to measurement of ^{3}H-labelled thymidine incorporation into the lymph node cells. No signs of systemic toxicity were noticed. No increase in stimulation index (SI) of cell count by a factor of greater than 1.5 or of ^{3}H-labelled thymidine incorporation by a factor of greater than or equal to 3 was observed after ametoctradin exposure. It was concluded that ametoctradin, at concentrations up to 50%, does not show a skin sensitizing effect in the murine local lymph node assay (Gamer & Landsiedel, 2007c).

In a second murine local lymph node assay, groups of five female CBA/J mice were treated with either 50% w/w preparations of ametoctradin (purity 100%, batch F15101209/022) or the vehicle alone. Higher concentrations were not technically achievable. The vehicle was a mixture of acetone and olive oil. A group of five female CBA/J mice treated with 25% α-hexylcinnamaldehyde in the acetone/olive oil vehicle was included as a positive control. The respective test substance preparation, vehicle control or positive control (25 µl per ear) was applied to the dorsum of both ears of each test group animal for 3 consecutive days. On day 5, the mice received 0.74 MBq of ^{3}H-labelled thymidine in 250 µl of sterile saline by intravenous injection into a tail vein. The animals were sacrificed about 5 hours after ^{3}H-labelled thymidine injection. From each animal, ear thickness, ear weight and lymph node weight were determined, in addition to measurement of ^{3}H-labelled thymidine incorporation into lymph node cells. No signs of systemic toxicity were observed. When applied as a 50% preparation in acetone/olive oil vehicle, no increase in SI by a factor of greater than 1.5 or of ^{3}H-labelled thymidine incorporation by a factor of greater than or equal to 3 was observed. It was concluded that ametoctradin, in a 50% preparation, is not a skin sensitizer (Remmele & Landsiedel, 2010).

In a dermal sensitization study using the Magnusson and Kligman maximization test, ametoctradin (purity 99.3%, batch COD-000748) was tested using 10 Dunkin-Hartley guinea-pigs. The control group consisted of five animals. The intradermal induction step was performed on day 0 with a 5% test substance preparation in 1% aqueous carboxymethylcellulose solution or a 5% test substance preparation in Freund's complete adjuvant/0.9% aqueous sodium chloride solution (1:1). The topical induction was performed on day 7 with a 60% test substance preparation in 1% aqueous carboxymethylcellulose solution (highest concentration technically achievable). For the topical challenge, on day 21, the 60% test substance preparation in 1% aqueous carboxymethylcellulose solution was applied. The intradermal induction caused moderate and confluent to intense erythema. Moreover, swelling was observed at the injection sites in all test group animals. After topical induction, incrustation (partially open) could be observed, in addition to moderate and confluent erythema and swelling in all test group animals. After topical challenge, discrete or patchy erythema was noted in two test group animals after 24 hours. No skin findings were observed in any of the test group animals 48 hours after removal of the patch. It was concluded that ametoctradin does not have a sensitizing effect on the skin in the maximization test (Gamer & Landsiedel, 2007d; Gamer, 2009).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 90-day feeding study in C57BL/6NCrl mice, ametoctradin (purity 99.8%, batch COD-000567) was administered to groups of mice (10 of each sex) at a dietary concentration of 0, 500, 2000 or 6000 parts per million (ppm) (equal to 0, 100.8, 370.3 and 1118.8 mg/kg bw per day for males and 0, 167.6, 596.6 and 2086.5 mg/kg bw per day for females, respectively). Feed consumption and body weight were determined weekly. Clinical observation was undertaken at least once a day. Clinical biochemical and haematological examinations were performed towards the end of the administration period. Cell proliferation (S-phase response) was evaluated in the liver, kidney and urinary bladder using osmotic minipumps with 5-bromo-2′-deoxyuridine (BrdU) implanted in all animals 7 days before necropsy.

The mortality incidence, clinical signs, feed consumption and body weight were not affected after dietary exposure to ametoctradin. No treatment-related changes were observed in haematological

and clinical chemistry parameters, organ weights, pathological findings or cell proliferation in liver, kidney and urinary bladder.

The no-observed-adverse-effect level (NOAEL) was 6000 ppm (equal to 1118.8 mg/kg bw per day for males and 2086.5 mg/kg bw per day for females), the highest dose tested (Kaspers et al., 2007b).

Rats

In a short-term study of toxicity, Wistar rats (10 of each sex per group) were dosed with ametoctradin (purity 99.8%, batch COD-000567) for at least 90 days at a dietary concentration of 0, 1500, 5000 or 15 000 ppm (equal to 0, 105.8, 358.3 and 1083.2 mg/kg bw per day for males and 0, 123.3, 415.8 and 1235.1 mg/kg bw per day for females, respectively). Rats were observed for clinical signs at least once a day, whereas body weight and feed consumption were determined weekly. Ophthalmoscopic observations were carried out once prior to treatment and on day 77. Neurobehavioural screening, consisting of functional observational battery and motor activity examinations, was performed at the end of the study. Clinical biochemistry and haematological examinations were performed towards the end of the administration period. At necropsy, the weights of selected organs were recorded, and the organs were assessed by gross examination and examined histopathologically. Seven days prior to necropsy, osmotic minipumps subcutaneously filled with BrdU were implanted for S-phase response determination in the liver, kidney, urinary bladder and thyroid. Labelling indices were determined in control and high-dose animals.

There were no treatment-related changes in mortality, clinical signs of toxicity, body weight, feed consumption, ophthalmoscopic examination, neurobehavioural end-points, haematological parameters and urine analysis throughout the study at any dose level. In addition, there were no substance-related effects on the S-phase response in the tissues examined. For the blood chemistry analysis, a non-significant increase in triglycerides was noted for males in the high-dose group (144% of control), which was within the historical control range and therefore considered to be unrelated to treatment. In high-dose males, there was a slight increase in liver weight by 10% (statistically significant relative to body weight). Because there were no corresponding histopathological changes found in the liver, this increase in liver weight is considered to be not treatment related or toxicologically relevant.

The NOAEL was 15 000 ppm (equal to 1083.2 mg/kg bw per day for males and 1235.1 mg/kg bw per day for females), the highest dose tested (Kaspers et al., 2007a).

Dogs

In a 90-day dietary study, groups of purebred Beagle dogs (five of each sex per dose) were dosed with ametoctradin (purity 99.3%, batch COD-000748) at a dietary concentration of 0, 3000, 10 000 or 30 000 ppm (equal to 0, 93, 299 and 912 mg/kg bw per day for males and 0, 100, 330 and 1006 mg/kg bw per day for females, respectively). Feed consumption was determined each working day, and body weight once a week. At least once a day, animals were observed for signs of toxicity. Ophthalmoscopic examinations were carried out 13 days prior to treatment and at the end of the study period. Clinical chemistry, haematological examinations and urine analyses were carried once before treatment, after 6 weeks and towards the end of the substance administration. After necropsy, all animals were subjected to gross pathological assessment, followed by histopathological examination.

No mortality or changes in feed consumption or body weight were noted. Regarding clinical observation, ophthalmoscopy, clinical chemistry, haematological examination and urine analyses, dietary exposure to ametoctradin at concentrations up to 30 000 ppm did not induce any treatment-related changes. A statistically significant increase in absolute thyroid weight was noted at 10 000 and 30 000 ppm in females (28.7% and 26.3%, respectively). In addition, a non-significant decrease in mean uterine weight was found in females at the high dose (37.8%). As there were no corresponding histological findings, these changes in organ weights were regarded to be incidental and not related to the test substance.

The NOAEL was 30 000 ppm (equal to 912 mg/kg bw per day for males and 1006 mg/kg bw per day for females), the highest dose tested (Hempel et al., 2007).

In a 1-year dietary toxicity study, ametoctradin (purity 99.3%, batch COD-000748) was administered to Beagle dogs (five of each sex per dose) at 0, 3000, 10 000 or 30 000 ppm (equal to 0, 84, 273 and 848 mg/kg bw per day for males and 0, 85, 305 and 936 mg/kg bw per day for females, respectively). The dogs were examined at least once a day for signs of toxicity. Feed consumption was determined each working day, and body weight on a weekly basis. Ophthalmoscopic examinations were performed once before the start and at the end of the study. Haematological and clinical chemistry parameters were determined at the end of the study period. Urine analyses were carried out before the start of treatment and after about 3, 6 and 12 months. After necropsy, selected organs and tissues were examined by gross pathology and histopathology.

No substance-related mortality was observed upon dietary exposure to ametoctradin for 1 year. Although decreased feed intake was not observed on all days on which it was determined, overall, during the whole study period, feed intake was lower in low-dose females than in controls. In addition, for mid-dose males, a lower feed intake was noted on several occasions during the exposure period. Body weight gain was increased in mid-dose females on several occasions. As no significant effect on body weight was observed, these effects were considered not to be toxicologically relevant. Ophthalmoscopic examinations revealed no substance-related findings. White blood cell count was statistically significantly reduced in mid-dose males only. Tubular degeneration of the testes and epididymides (aspermia and oligospermia) was noted in high-dose males. However, the effects on the epididymides were not observed in the high-dose males in the 90-day dietary toxicity study (see above), but were observed in one male of the control and intermediate-dose groups. Tubular degeneration was observed in the 90-day dog study (see above) in one male of each dose group, but in a comparison of the severity, there was no dose–response relationship. This indicates that the findings in the testes and epididymides can be considered incidental and not test substance related.

The NOAEL was 30 000 ppm (equal to 848 mg/kg bw per day for males and 936 mg/kg bw per day for females), the highest dose tested (Hempel et al., 2008).

(b) Dermal application

In a 28-day dermal toxicity study, ametoctradin (purity 99.3%, batch COD-000748) was applied to the shaved, intact dorsal skin of Wistar rats (10 of each sex per dose) at a dose of 0, 100, 300 or 1000 mg/kg bw per day under semi-occlusion. The vehicle was 1% aqueous carboxymethylcellulose. Animals were examined at least once a day for signs of toxicity. Body weight and feed consumption were determined on a weekly basis. Ophthalmoscopy was carried out once prior to the start of the study and once at the end of the administration period. A functional observational battery was performed on all animals at the end of the study. Urine analyses, clinical biochemistry as well as haematological examinations were carried out at the end of the study. All animals underwent gross pathological and histopathological examinations.

No substance-related effects on mortality, clinical signs, feed consumption or ophthalmological examinations were observed. The neurobehavioural studies did not reveal any treatment-related effects. Males in the mid- and high-dose groups showed a lower body weight gain on days 21 and 28, resulting in slightly lower body weights on those days (approximately 5% lower). Haematological and urine analyses parameters were unchanged. Clinical biochemistry parameters showed an increase in bilirubin levels in males at 100, 300 and 1000 mg/kg bw per day (109%, 113% and 121% of controls, respectively). As these changes were not accompanied by other corroborative changes (e.g. changes in red blood cells), this finding was not considered to be toxicologically relevant. The absolute weight of the brain in high-dose males was statistically significantly decreased (4%) compared with controls. The statistically significant change in absolute brain weight is regarded as not treatment related, as no relative weight changes or histopathological findings were noted in the brain. The relative weights of the adrenal glands in the males at 100 and 1000 mg/kg bw per day and

the relative weight of the liver in the males at 300 mg/kg bw per day were statistically significantly increased (18.2% in both dose groups for relative adrenal gland weight and 4.9% for relative liver weight). As the effects were without a dose–response relationship and the organs showed no corresponding histopathological changes, the findings were not considered to be toxicologically relevant.

The NOAEL in both males and females was 1000 mg/kg bw per day, the highest dose tested (Kaspers et al., 2007c; Kaspers, 2009).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a carcinogenicity study, C57BL/6 J Rj mice (50 of each sex per group) received ametoctradin (purity 99.3%, batch COD-000748) at a dietary concentration of 0, 60, 600 or 6000 ppm (equal to 0, 10.6, 104.2 and 1099 mg/kg bw per day for males and 0, 15.2, 154.1 and 1543 mg/kg bw per day for females) for 18 months. Observations for mortality and clinical signs were carried out at least once a day. Feed consumption and body weight were monitored weekly during the first 13 weeks and thereafter at 4-week intervals until the end of the administration period. Haematological and clinical chemistry examinations were performed at the end of the study. At necropsy, the weights of selected organs were recorded for all mice. All major organs were examined by gross microscopy as well as histopathologically.

No substance-related changes in mortality, clinical signs or feed consumption were noted. A slight, but statistically significant, decrease in body weight (gain) was observed in high-dose males only. As the decrease in body weight was less than 10%, this effect was not considered to be toxicologically relevant. At the end of dosing, a slightly higher incidence of different plasma changes in the lymphocytes of the high-dose females was observed (8/47 mice compared with 3/46 mice in the controls). However, the counts of changed lymphocytes per dosed mouse were between 1 and 2 per 100 cells, whereas 2–6 changed lymphocytes per 100 cells were found in control mice. Furthermore, the relevance of these haematological effects in such old mice, with a high standard deviation, can be questioned. At the end of the administration period, slightly more females in the 6000 ppm dose group with 1–2 metamyelocytes per 100 cells were found (5/47 mice compared with 0/46 mice in the controls). However, as a few mice with single metamyelocytes were also found in the controls of both sexes after 12 months as well as in male animals at the end of the administration period, the effect can be regarded as incidental and not substance related. Both the absolute and relative kidney weights were significantly reduced (−8.8% and −8.2%, respectively) in high-dose males. In high-dose females, absolute liver weight was increased by 11% compared with controls. As the observed weight changes were only slight and not accompanied by other toxic effects, they were not considered to be toxicologically relevant. There were no treatment-related effects on the incidence of neoplastic or non-neoplastic lesions.

Based on the absence of toxicologically relevant effects in the dose groups tested, the NOAEL was set at 6000 ppm (equal to 1099 mg/kg bw per day for males and 1543 mg/kg bw per day for females), the highest dose tested. Ametoctradin showed no carcinogenic potential in mice (Kamp et al., 2008).

Rats

In a 2-year combined chronic toxicity and carcinogenicity study, ametoctradin (purity 99.1–99.3%, batches COD-000682 and COD-000748) was administered to Wistar rats (50 of each sex per group). Dietary concentrations of 0, 150, 1500 and 15 000 ppm (raised to 20 000 ppm on day 308 and to 22 500 ppm on day 336 in males) were used, equal to a daily test substance intake over the entire administration period of 0, 6.9, 69.9 and 870.7 mg/kg bw per day for males and 0, 9.6, 95.0 and 979.3 mg/kg bw per day for females, respectively. As the test substance concentration in feed was raised

twice for high-dose males during the study, the intakes at the beginning and end of the study and around the increase on days 308 and 336 are indicated in Table 6.

Additional groups of rats (10 of each sex per dose) were treated with ametoctradin for interim sacrifice after 1 year. Feed consumption and body weight were observed weekly for the first 13 weeks and at 4-week intervals thereafter. The animals were examined for signs of toxicity or mortality at least once a day. An ophthalmoscopic examination was carried out on all animals prior to treatment and at the end of the study. Urine analysis, clinical chemistry and haematological examinations were carried out in the interim sacrifice group at 3, 6 and 12 months. After necropsy, selected organs were weighed, and complete macroscopic and histopathological evaluations were conducted on all animals.

Table 6. Mean intake of ametoctradin in male rats in the 2-year study

Day	Mean test substance intake (mg/kg bw per day)		
	150 ppm	1500 ppm	15 000 ppm
7	14.5	144.0	1459.3
287	6.5	66.5	664.4
315[a]	6.3	64.2	855.9
336[a]	NC	NC	858.3
343	6.3	62.9	963.5
728	5.6	59.2	899.2

From Kaspers et al. (2008a); Buesen (2010a,b)
NC, not calculable
[a] Dietary concentration for high dose group males was raised from 15 000 to 20 000 ppm on day 308 and to 22 500 ppm on day 336.

No substance-related mortality or clinical signs of toxicity were observed during dietary exposure of the rats to ametoctradin. Body weight was significantly lower in high-dose males from day 357 (approximately 4%) until the end of the study (approximately 8%). Similarly, body weight gain was also significantly reduced. In high-dose females, significant decreases in body weight (maximally 7%) and body weight gain were noted throughout the study. However, no effect on feed consumption was observed in either sex. As the decreases in both body weight and body weight gain at the end of the study were small (< 10%), they were not considered toxicologically relevant. No substance-related effects were observed in ophthalmology, clinical chemistry, urine analysis or haematology. In males, an increase in mean absolute epididymides weight was observed in the satellite group at all doses (21.7%, 8.8% and 9.0% in low-, mid- and high-dose males, respectively), and an increase in relative epididymides weight was observed in the main group (13.2%, 13.7% and 10.6%, respectively). In addition, increases in relative kidney weight in all dosed males (14.2%, 10.8% and 8.1% in low-, mid- and high-dose males, respectively), in relative heart weight in low- and mid-dose males (10% and 6.3%, respectively) and in relative liver weight in mid- and high-dose males (4.8% and 7.2%, respectively) were noted. However, the weight increases were not dose related, were observed only in males and were not accompanied by histopathological findings. An increase in the incidence of dilution of the ductus choledochus was observed at 24 months in mid- and high-dose males (25.6% and 26.5% compared with 15.8% in controls) and in high-dose females (20% compared with 8.8% in controls). In the absence of corroborative findings in haematological parameters, clinical signs and histopathology, the effect was not considered to be adverse. No other substance-related non-neoplastic findings were observed.

There were no substance-related neoplastic findings.

The NOAEL was concluded to be 870.7 mg/kg bw per day for males and 979.3 mg/kg bw per day for females, the highest dose tested in both sexes. Ametoctradin was not carcinogenic in Wistar rats under the study conditions (Kaspers et al., 2008a; Buesen, 2010a,b).

2.4 Genotoxicity

The genotoxic potential of ametoctradin was evaluated in a battery of four in vitro and four in vivo studies (Table 7). Ametoctradin gave negative results in all these studies. Therefore, ametoctradin is unlikely to be genotoxic.

Table 7. Results of genotoxicity studies with ametoctradin

End-point	Test system	Concentration/dose	Purity; batch	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2*uvrA*; standard plate incorporation and preincubation assay	20–5000 µg/plate (±S9)	99.3%; COD-00748	Negative	Schulz & Landsiedel (2006)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2*uvrA*; standard plate incorporation and preincubation assay	33–5000 µg/plate (±S9)	100%; F15101209/022	Negative	Schulz & Landsiedel (2010a)
Chromosomal aberrations	V79 cells	−S9: 25–100 µg/ml (4 h) 25–200 µg/ml (18 h) +S9: 12.5–100 µg/ml (4 h)	99.5%; COD-000480	Negative	Schulz (2005)
Gene mutation (HPRT)	CHO-K1 cells	1st experiment: 31.3–1000 µg/ml (±S9) 2nd experiment: 25–300 µg/ml (±S9)	99.3%; COD-000748	Negative	Schulz & Landsiedel (2007a)
In vivo					
Chromosomal aberrations	Wistar rats, bone marrow cells	500–2000 mg/kg bw	99.5%; COD-000480	Negative	Engelhardt & Leibold (2005a); Landsiedel (2008)
Micronucleus formation	NMRI mice, bone marrow cells	500–2000 mg/kg bw	99.8%; COD-000567	Negative	Engelhardt & Leibold (2005b)
Micronucleus formation	NMRI mice, bone marrow cells	2000 mg/kg bw	100%; F15101209/022	Negative	Schulz & Landsiedel (2010b)
Unscheduled DNA synthesis	Wistar rats, primary hepatocytes	1000, 2000 mg/kg bw	99.5%; COD-000480	Negative	Honarvar (2005)

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a two-generation reproductive toxicity study, ametoctradin (purity 99.3%, batch COD-00748) was administered to groups of 25 male and 25 female Wistar rats continuously throughout the

study at dietary concentrations that were adjusted to obtain target dose levels of 0, 100, 300 and 1000 mg/kg bw per day. The actual dietary intakes for the F_0 and F_1 generations are shown in Table 8. At least 75 days after the beginning of treatment, F_0 animals were mated to form the F_1 generation. Groups of 25 males and 25 females, selected from F_1 pups to become the F_1 parental generation, were continued on the same dose as their parents post-weaning, and the breeding programme was repeated to produce an F_2 litter. The examinations of parental animals included monitoring of mortality, clinical signs, feed consumption and body weight. The pups were weighed on postnatal days 1, 4, 7, 14 and 21. A comprehensive evaluation of male and female reproductive systems was conducted, including evaluation of the estrous cycle, mating performance, sperm parameters, conception, gestation, parturition and lactation, as well as survival, growth and development of the offspring. All F_0 and F_1 parental animals were assessed for changes in organ weights, gross pathology and histopathology. After termination, all pups were examined externally and their organs were assessed macroscopically. The brain, spleen and thymus of one pup of each sex and litter from the F_1 and F_2 generations were weighed.

Table 8. Mean intake of ametoctradin at target dose levels in the two-generation study in rats

	Average ametoctradin intake (mg/kg bw per day)					
	F_0 generation			F_1 generation		
	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day
Males	94.4	282.6	944.0	93.6	280.2	938.7
Females, premating	95.5	285.3	951.3	96.8	291.3	965.3
Females, gestation period	101.2	308.7	1044.9	100.8	297.6	1043.0
Females, lactation period	95.8	296.7	1010.9	93.7	285.3	954.9

In F_0 and F_1 animals, mortality, feed consumption and body weight were not affected by treatment, and no treatment-related clinical signs were observed. In addition, there were no treatment-related effects on reproductive parameters. Relative liver and kidney weights were significantly increased in F_0 and F_1 animals in all dose groups (maximum increase of 10%). However, owing to the lack of a clear dose–response relationship and the absence of corresponding histopathological findings, these effects were not considered to be toxicologically relevant. In F_0 females, significant decreases in uterine weight (16% and 18%, respectively) were noted for the mid- and high-dose groups. In F_1 females, a similar significant decrease in uterine weight was observed in high-dose animals only. However, a high variation in uterine weight was apparent. Furthermore, the observed weight decreases were within the historical control range and not corroborated by any histopathological findings or functional impairment. In F_1 pups, perinatal and postnatal survival and post-weaning development until sexual maturity were unaffected by the test substance at any dose tested. No substance-related changes were observed in litter size, pup viability, sex ratio or clinical signs of the F_2 pups.

The NOAEL was 939 mg/kg bw per day, the highest dose tested, with regard to systemic toxicity, fertility and reproductive performance in parental F_0 and F_1 rats as well as prenatal and postnatal developmental toxicity in their offspring (Schneider et al., 2008a).

(b) Developmental toxicity

Rats

In a prenatal developmental toxicity study, groups of 25 time-mated female Wistar rats were dosed with ametoctradin (purity 99.8%, batch COD-000567) by gavage at 0, 100, 300 or 1000 mg/kg bw per day on gestation day (GD) 6 to GD 19. Mortality and clinical symptoms were examined at least once a day. All animals were weighed on GDs 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20. Feed

consumption was determined on the same days as body weight, with the exception of GD 0. On GD 20, all females were sacrificed and underwent gross pathological examination. Examinations included uterine weight and numbers of corpora lutea, implantation sites, live and dead fetuses, and resorptions. Each fetus was weighed, sexed and examined macroscopically for any external findings. Approximately half of the fetuses were preserved in Harrison's fluid and examined for any visceral findings. The remaining fetuses were eviscerated, skinned, fixed in ethyl alcohol and then stained with alizarin red and alcian blue for skeletal examination.

There were no substance-related effects on mortality, clinical signs, feed consumption or body weight in the maternal animals. The number of corpora lutea, the number of viable fetuses and fetal weight were unaffected by ametoctradin at all dose levels. There were no treatment-related changes observed in the external and visceral examinations. However, several skeletal variations were recorded. A significant increase in the incidence of incomplete ossification of the skull was observed at the high dose (9.4% affected fetuses per litter), but it remained within the range of historical controls (0–11%). In high-dose animals, a significant increase in the incidence of misshaped tuberositas deltoidea was also noted (3.8%), but it was within the range of the historical controls (0–6.8%). A significant increase in the incidence of bipartite ossification of thoracic centrum was observed in the mid-dose group only. Considering the lack of a dose–response relationship, this effect was not considered to be toxicologically relevant. The incidence of misshapen sternebrae was also significantly increased at the high dose (53.1% affected fetuses per litter compared with 38.8% in the controls and 7.7–52.1% in the historical controls). Considering the high incidence in control animals, 53.1% is only slightly higher than the upper limit of the historical control range and therefore considered incidental and of minor toxicological relevance.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day. As all the skeletal variations were within the historical control ranges or only marginally above the upper limit of the range, these findings were considered to be incidental. Therefore, the NOAEL for developmental toxicity was set at 1000 mg/kg bw per day, the highest dose tested (Schneider et al., 2006).

Rabbits

Ametoctradin (purity 99.3%, batch COD-000748) was tested for its prenatal developmental toxicity in Himalayan rabbits. Groups of 25 female inseminated Himalayan rabbits were administered the test substance by gavage at a dose of 0, 100, 300 or 1000 mg/kg bw per day from GD 6 through GD 28. Mortality and clinical symptoms were checked on a daily basis. Feed consumption and body weight were observed regularly throughout the study period. On GD 29, the surviving females were sacrificed and assessed by gross pathological examination. For each animal, uterine weight and the numbers of corpora lutea, early and late resorptions, and live and dead fetuses were recorded. The fetuses were removed from the uterus, sexed, weighed and further evaluated for any external, soft tissue and skeletal findings, including cartilage staining.

Ametoctradin showed no effect on mortality or clinical signs. Feed consumption was slightly higher in high-dose females, and consequently a slightly higher final body weight was observed. However, maternal body weights were still within the range of the historical controls. There were no treatment-related effects on the numbers of corpora lutea, preimplantation and post-implantation losses or viable fetuses, sex ratio or fetal weight. In addition, there were no significant changes in external observations in any of the dose groups. Visceral examination showed a slight increase in the incidence of haemorrhagic ovary (1.4% affected fetuses per litter) and in the incidence of blood coagulum around the urinary bladder (2.8% affected fetuses per litter). As the observed incidences were within the range of the historical controls (0–3.8% for haemorrhagic ovary and 0–8.3% for blood coagulum around the urinary bladder), the effects were considered incidental. The incidence of soft tissue variations was increased in the mid-dose group only (15.2% affected fetuses per litter compared with 5.7% in controls), mainly due to an increase in the incidence of absent lung lobe (14% compared with 3.8% in controls). However, the effect was within the same range as the historical control (3.3–33.6%). An increase in the incidence of supernumerary 13th rib was noted at the high dose (7.0%

affected fetuses per litter). However, the observed incidence was within the historical control range (0–10.4%) and therefore considered to be not adverse.

The NOAEL for maternal and prenatal developmental toxicity was 1000 mg/kg bw per day, the highest dose tested. No indications for teratogenicity were observed in any test group (Schneider et al., 2008b).

2.6 Special studies

(a) Neurotoxicity

The acute neurotoxicity of ametoctradin (purity 99.3%, batch COD-000748) was investigated in Wistar rats (10 of each sex per group) after a single oral administration by gavage at a dose of 0, 125, 500 or 2000 mg/kg bw. The animals were observed for up to 2 weeks after dosing. General health was observed daily. Body weight, functional observational battery and motor activity measurements were carried out on days −6, 0, 7 and 14. At the end of the study, five animals of each sex per dose were fixed by in situ perfusion and subjected to neuropathological examination.

No treatment-related changes were observed in mortality, clinical symptoms or body weight. In addition, functional observational battery and motor activity measurements were not affected by ametoctradin at doses up to 2000 mg/kg bw, nor were any substance-related neuropathological changes observed.

The NOAEL for acute neurotoxicity was 2000 mg/kg bw, the highest dose tested (Kaspers et al., 2009a).

In a subchronic neurotoxicity study, ametoctradin (purity 99.3%, batch COD-000748) was administered to Wistar rats (10 of each sex per dose) at a dietary concentration of 0, 1500, 5000 or 15 000 ppm (equal to doses of 0, 89.4, 299.9 and 921.2 mg/kg bw per day for males and 0, 104.5, 349.7 and 1077.2 mg/kg bw per day for females, respectively) for a period of 3 months. Mortality and signs of toxicity were examined at least once a day. Feed consumption was determined once a week. Body weight was measured weekly and on days when functional observational battery tests were performed. Functional observational batteries and measurement of motor activity were carried out on days −7, 1, 22, 50 and 85. Five animals of each sex per dose were fixed by in situ perfusion and subjected to neuropathological examination.

Ametoctradin at dietary concentrations up to 15 000 ppm did not affect mortality, clinical examinations or body weight. No changes with regard to the functional observational battery or motor activity measurements were observed. In addition, no treatment-related effects were noted in the neuropathological examinations.

Under the conditions of this study, the NOAEL for neurotoxicity was 15 000 ppm (equal to 921.2 mg/kg bw per day for males and 1077.2 mg/kg bw per day for females), the highest dose tested (Kaspers et al., 2009b).

(b) Immunotoxicity

Ametoctradin (purity 99.3%, batch COD-000748) was administered to groups of eight female C57BL/6 J Rj mice at a dose of 0, 500, 2000 or 6000 ppm (equal to 0, 160.0, 603.5 and 1955.7 mg/kg bw per day, respectively) in the diet over a period of 4 weeks. In addition, a positive control group received cyclophosphamide monohydrate by gavage at a dose of 12 mg/kg bw per day. Feed consumption and body weight were determined weekly. The animals were examined for mortality and signs of toxicity at least once a day. Lymphocyte count, determinations of lymphocyte subpopulations, measurements of sheep red blood cell immunoglobulin M (IgM) antibody titres as well as natural killer (NK) cell activity tests were carried out at the end of the study. In addition, all animals were assessed by gross pathological examination, and the organ weights of spleen and thymus were measured.

There were no treatment-related effects, and no mortality or clinical signs were observed after ametoctradin treatment. Moreover, feed consumption and body weight were unchanged in animals treated with ametoctradin compared with controls. No changes in total lymphocyte counts or in absolute and relative lymphocyte subpopulation counts were measured in the dosed animals. In addition, no test substance–related changes were observed in NK cell activity, in the sheep red blood cell IgM titres or in the pathological examinations.

The NOAEL was 6000 ppm (equal to 1955.7 mg/kg bw per day), the highest dose tested (Kaspers et al., 2009c).

3. Studies on metabolites

3.1 M650F02

4-(7-Amino-5-ethyl[1,2,4]triazolo[1,5-a]pyrimidin-6-yl)propanoic acid (company code M650F02) was tested in two genotoxicity tests, with negative results (Table 9).

Table 9. Results of genotoxicity studies with M650F02

End-point	Test system	Concentration/ dose	Purity; batch	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2*uvrA*; standard plate incorporation and preincubation assay	24–6000 µg/plate (±S9)	87.4%; L71-56	Negative	Engelhardt & Hellwig (2005a); Engelhardt (2006a)
In vivo					
Micronucleus formation	NRMI mice, bone marrow cells	575–2300 mg/kg bw	87.4%; L71-56	Negative	Engelhardt & Mellert (2006)

S9, 9000 × g supernatant fraction of rat liver homogenate

3.2 M650F03

In a 90-day oral toxicity study, Wistar rats (10 of each sex per group) received 7-amino-5-ethyl[1,2,4]triazolo[1,5-a]pyrimidin-6-acetic acid (company code M650F03) (purity 97.2%, batch L71-154) at a dietary concentration of 0, 1500, 5000 or 15 000 ppm (equal to 0, 89.5, 298.8 and 942.6 mg/kg bw per day for males and 0, 107.1, 348.6 and 1093.6 mg/kg bw per day for females, respectively). Feed consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Functional observational battery tests, measurement of motor activity, clinical biochemistry, haematological examinations and urine analyses were performed towards the end of the treatment period. Ophthalmoscopic examinations were conducted before treatment and towards the end of the administration period. All animals underwent gross pathological and histopathological examinations.

No mortalities were observed in any of the dose groups. Body weight, feed consumption, functional observational battery, ophthalmoscopy, haematology, clinical biochemistry and urine analysis were unchanged after exposure to dietary concentrations of ametoctradin up to 15 000 ppm. Regarding motor activity, a slight decrease was noted in the overall beam interrupts (interval 1–12) in high-dose males. As no other effects on motor activity were observed, this slight effect was considered not substance related or toxicologically relevant. No treatment-related adverse effects on clinical pathology parameters were observed.

The NOAEL was 15 000 ppm (equal to 942.6 mg/kg bw per day for males and 1093.6 mg/kg bw per day for females), the highest dose tested (Kaspers et al., 2008b).

M650F03 was tested in a battery of in vitro and in vivo genotoxicity studies (Table 10). M650F03 was negative in all tests.

Table 10. Results of genotoxicity studies with M650F03

End-point	Test system	Concentration/dose	Purity; batch	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2*uvrA*; standard plate incorporation (1st experiment) and preincubation assay (2nd and 3rd experiments)	1st experiment: 20–5000 µg/plate (±S9) 2nd experiment: 4–2500 µg/plate (±S9) 3rd experiment: 500–4000 µg/plate (±S9)	98.1%; L71-55	Negative	Engelhardt & Hellwig (2005b); Engelhardt (2006b)
Gene mutation (HPRT)	CHO-K1 cells	1st experiment −S9: 800–1800 µg/ml +S9: 100–1000 µg/ml 2nd experiment: −S9: 1400–1600 µg/ml +S9: 500–1000 µg/ml	98.1%; L71-55	Negative	Engelhardt & Landsiedel (2006)
Chromosomal aberration	V79 cells	1st experiment: −S9: 275–2200 µg/ml (18 h) +S9: 137.5–2200 µg/ml (18 h) 2nd experiment: −S9: 275–2200 µg/ml (18 h) −S9: 1100–2200 µg/ml (28 h) +S9: 550–2200 µg/ml (28 h)	98.1%; L71-55	Negative	Schulz & Landsiedel (2007b)
In vivo					
Micronucleus formation	NMRI mice, bone marrow	500–2000 mg/kg bw	98.1%; L71-55	Negative	Engelhardt (2006c); Engelhardt & Hellwig (2006)

S9, 9000 × *g* supernatant fraction of rat liver homogenate

3.3 M650F04

7-Amino-5-ethyl[1,2,4]triazolo[1,5-a]pyrimidin-6-carboxylic acid (company code M650F04) (purity 95.8%, batch L71-126) was administered to Wistar rats (10 of each sex per group) at a dietary concentration of 0, 1500, 5000 or 15 000 ppm (equal to 0, 96.9, 317.9 and 1033.5 mg/kg bw per day for males and 0, 114.9, 418.0 and 1161.4 mg/kg bw per day for females, respectively) for 3 months. Feed consumption and body weight were recorded on a weekly basis. The animals were examined for signs of toxicity or mortality at least once a day. A functional observational battery, measurement of

motor activity, clinical biochemistry, haematological examinations and urine analyses were carried out towards the end of the study. Ophthalmoscopic examinations were performed before and towards the end of the administration period. After termination, all animals underwent gross pathological and histopathological examinations.

No mortalities were observed in any of the treatment groups throughout the study, nor were any treatment-related clinical signs noted. Increased water consumption was observed in high-dose males (20–50%) and females (10–30%). However, the increase in water consumption was not accompanied by increased urine production. No treatment-related changes were observed in feed consumption, haematology, clinical chemistry, ophthalmoscopy or urine analysis. In motor activity tests, high-dose females showed a slight decrease in the overall beam interrupts (interval 1–12) compared with controls. However, no other effects on motor activity were found. A slight increase in absolute brain weight was observed in low-dose (3%) and high-dose males (2%). The absence of a dose–response relationship and the lack of corroborating histopathological findings suggested that the observed effect was not toxicologically relevant. Relative liver weight was slightly increased in males in the low-dose (5%), mid-dose (6%) and high-dose groups (8%). In high-dose females, a slight decrease in relative spleen weight (9%) was observed. As the observed effects were only slight (< 10%) and not accompanied by histopathological changes or effects on absolute weight, these slight weight changes were regarded as not toxicologically relevant. Histopathological findings in the lungs of five high-dose male animals included minimal alveolar histiocytosis compared with a slight alveolar histiocytosis in the lungs of only one control male animal. Considering that six control females as well as four high-dose females showed this finding, the effect was regarded as a spontaneous finding.

The NOAEL was 15 000 ppm (equal to 1033.5 mg/kg bw per day for males and 1161.4 mg/kg bw per day for females), the highest dose tested (Kaspers et al., 2008c).

The potential genotoxicity of M650F04 was investigated in three in vitro tests, with negative results. The studies are summarized in Table 11.

4. Observations in humans

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of ametoctradin. Thus, the medical monitoring programme is designed as a general health checkup, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments. The surveillance programme includes a general physical examination, including neurological status, red and white blood cell counts and liver enzymes. Adverse health effects suspected to be related to ametoctradin exposure have not been observed.

No human cases of intoxication or poisoning deriving from ametoctradin are known to BASF SE.

Neither data on exposure of the general public nor epidemiological studies are available for BASF SE, nor is BASF SE aware of any epidemiological studies performed by third parties.

Table 11. Results of genotoxicity studies with M650F04

End-point	Test system	Concentration/dose	Purity; batch	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2*uvrA*; standard plate incorporation (1st experiment) and preincubation assay (2nd and 3rd experiments)	1st experiment: 20–5000 µg/plate (±S9) 2nd experiment: 20–5000 µg/plate (±S9) 3rd experiment: TA98: 2–500 µg/plate (±S9) TA100: 10–2500 µg/plate (±S9) TA1535: 20–5000 µg/plate (−S9) TA1535: 8–2000 µg/plate (+S9) TA1537: 2–500 µg/plate (+S9) *E. coli*: 0.4–100 µg/plate (+S9)	99.2%; L74-106	Negative	Schulz & Landsiedel (2007c)
Gene mutation (HPRT)	CHO-K1 cells	1st experiment: 131.3–2100 µg/ml (±S9) 2nd experiment: −S9: 500–2100 µg/ml +S9: 131.3–2100 µg/ml	99.2%; L74-106	Negative	Schulz & Hellwig (2006)
Chromosomal aberrations	V79 cells	1st experiment: 131.3–2100 µg/ml (±S9) (4 h exposure, 18 h sampling time) 2nd experiment: 131.3–2100 µg/ml (−S9) (18 h exposure, 18 h sampling time) 525.0–2100 µg/ml (−S9) (18 h exposure, 28 h sampling time) 131.2–2100 µg/ml (+S9) (4 h exposure, 28 h sampling time)	99.2%; L74-106	Negative	Schulz & Landsiedel (2007d)

S9, 9000 × *g* supernatant fraction of rat liver homogenate

Comments

Biochemical aspects

Following oral administration, ^{14}C-labelled ametoctradin underwent limited and saturable absorption from the gastrointestinal tract, but was quite widely distributed. Maximum plasma concentrations were observed within 1–2 hours after administration, and initial half-lives ranged from 2 to 3 hours. The AUC values indicate that internal exposure was not different in males and females. Excretion of ametoctradin occurred rapidly and independently of sex. Most of the administered dose (91–110%) was recovered within 168 hours after a single low or high dose and repeated high doses, with faeces as the main elimination route. Based on the amount of radioactivity excreted via bile and urine, the bioavailability of ametoctradin in rats was calculated to be about 40% of the administered dose at 50 mg/kg bw and about 20% of the applied dose at 500 mg/kg bw. The parent compound is metabolized by terminal oxidation of the octyl side-chain to the respective carboxylic acid (M650F09), with subsequent degradation of the carboxylic side-chain to give M650F06 and M650F01. In addition, conjugation of the respective oxidized side-chain with taurine and/or glucuronic acid occurs, leading to metabolites M650F10 (taurine conjugate of M650F09), M650F11

(glucuronic acid conjugate of M650F06) and M650F12 (taurine conjugate of M650F06). Also, a minor metabolic step leads to the formation of M650F05 (ω-hetarylpentanoic acid). Several metabolites of ametoctradin were found in liver, kidneys, plasma and bile, with metabolite M650F06 being the most abundant.

Toxicological data

Ametoctradin has low acute oral and dermal toxicity (LD_{50} values > 2000 mg/kg bw) and low toxicity by inhalation (LC_{50} > 5.4 mg/l). No skin or eye irritation was observed after ametoctradin exposure. Ametoctradin was not a sensitizer in a Magnusson and Kligman maximization test or in the murine local lymph node assay.

In repeated-dose toxicity studies in mice, rats and dogs, no consistent toxicological findings were evident in any of the species at any dose tested up to the limit dose (around 1000 mg/kg bw per day) or after any study duration. Ametoctradin was extensively tested in a comprehensive set of current guideline studies, including short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, studies of reproductive and developmental toxicity, neurotoxicity studies and an immunotoxicity study.

In the long-term studies of toxicity and carcinogenicity, no treatment-related changes in tumour incidence were observed.

The Meeting concluded that ametoctradin was not carcinogenic in mice or rats.

Ametoctradin was tested for genotoxicity in an adequate range of in vitro and in vivo studies. No evidence for genotoxicity was observed in any of these tests.

The Meeting concluded that ametoctradin was not genotoxic.

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

The toxicity of several soil metabolites of ametoctradin was examined. Ninety-day dietary toxicity studies in rats were performed with metabolites M650F03 and M650F04, metabolites that were not found in the rat. In the 90-day dietary toxicity studies, no adverse effects were observed after exposure to either M650F03 or M650F04 up to the limit dose (i.e. 15 000 ppm, equivalent to about 1000 mg/kg bw per day). The genotoxic potential of three soil metabolites, M650F02, M650F03 and M650F04, was tested in several in vitro and in vivo studies. All were negative for genotoxicity.

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

Toxicological evaluation

From the animal studies with ametoctradin, no adverse effects were observed at or near the limit dose of approximately 1000 mg/kg bw per day. The Meeting concluded that it was not necessary to establish an acceptable daily intake (ADI) for ametoctradin. This was based on a reasonable estimate of a likely maximal intake of the residues of a pesticide arising from the daily diet. In the 2004 JMPR report (Annex 1, reference 101) and in more detail in the publication by Solecki et al. (2005) on guidance on setting acute reference doses (ARfDs), a maximum cut-off of 5 mg/kg bw for the ARfD was suggested, based on food consumption estimates and maximum residue levels in foods. This cut-off would equate to a NOAEL of 500 mg/kg bw per day in an animal study, with the application of the default uncertainty factor of 100. A similar principle was considered by the Meeting to be applicable in setting an extreme upper bound for the ADI, noting that the long-term daily dietary exposure for the residues of a particular pesticide will be less than the international estimate of short-term dietary intake (IESTI) for the residues of that pesticide. A cut-off for the ADI could be refined, taking into account long-term high-level consumption.

The Meeting concluded that it was not necessary to establish an ARfD for ametoctradin in view of the absence of acute toxicity or any other effect that could be elicited by a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	6000 ppm, equal to 1099 mg/kg bw per day[b]	—
		Carcinogenicity	6000 ppm, equal to 1099 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	15 000–22 500 ppm, equal to 871 mg/kg bw per day[b]	—
		Carcinogenicity	15 000–22 500 ppm, equal to 871 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	939 mg/kg bw per day[b]	—
		Offspring toxicity	939 mg/kg bw per day[b]	—
		Reproductive toxicity	939 mg/kg bw per day[b]	—
	Developmental toxicity study[c]	Maternal toxicity	1000 mg/kg bw per day[b]	—
		Embryo and fetal toxicity	1000 mg/kg bw per day[b]	—
	Acute neurotoxicity study[c]	Neurotoxicity	2000 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity study[c]	Maternal toxicity	1000 mg/kg bw per day[b]	—
		Embryo and fetal toxicity	1000 mg/kg bw per day[b]	—
Dog	One-year study of toxicity[a]	Toxicity	30 000 ppm, equal to 848 mg/kg bw per day[b]	—

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

Estimate of acceptable daily intake for humans

Unnecessary

Estimate of acute reference dose

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Approximately 20% at high dose (500 mg/kg bw) and 40% at low dose (50 mg/kg bw)
Dermal absorption	No information on the pure active substance
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid and complete
Metabolism in animals	Limited; several metabolites, with M650F06 being most abundant
Toxicologically significant compounds in animals, plants and the environment	None
Acute toxicity	
Rat, LD_{50}, oral	> 2000 mg/kg bw per day
Rat, LD_{50}, dermal	> 2000 mg/kg bw per day
Rat, LC_{50}, inhalation	> 5.4 mg/l air (4 h, nose only)
Rabbit, dermal irritation	Non-irritant
Rabbit, ocular irritation	Non-irritant
Dermal sensitization	Not sensitizing (Magnusson & Kligman and local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	No adverse effects at the limit dose
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	No adverse effects at the limit dose
Carcinogenicity	No carcinogenic potential
Genotoxicity	
	No genotoxic potential
Reproductive toxicity	
Reproduction target/critical effect	No adverse effects at the limit dose
Developmental toxicity	
Developmental target/critical effect	No adverse effects at the limit dose
Neurotoxicity	
Acute neurotoxicity	No neurotoxicity at the limit dose
Other toxicological studies	
Immunotoxicity studies	No immunotoxicity at the limit dose
Studies performed on metabolites or impurities	M650F02: Not genotoxic
	M650F03: Not genotoxic; no effects at the limit dose in 90-day rat study
	M650F04: Not genotoxic; no effects at the limit dose in 90-day rat study
Medical data	
	Limited information; new compound

Summary

	Value	Study	Safety factor
ADI	Unnecessary	—	—
ARfD	Unnecessary	—	—

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