5.29 TIOXAZAFEN (311)

TOXICOLOGY

Tioxazafen is the ISO-approved common name for 3-phenyl-5-thiophen-2-yl-1,2,4-oxadiazole (IUPAC), which has the CAS number 330459-31-9.

Tioxazafen is a seed treatment nematicide for use on corn, soy and cotton. It appears to act through interaction with a nematode-specific insertion of the L3 subunit of the mitochondrial ribosome, leading to disruption of ribosomal translation in nematodes.

Tioxazafen has not previously been evaluated by JMPR and was reviewed by the present Meeting at the request of CCPR.

All critical studies contained statements of compliance with 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.

Biochemical aspects

Following the administration of a single oral [^{14}C]tioxazafen dose of 3 or 100 mg/kg bw to rats, absorption was rapid, with a peak concentration after 2 or 4 hours, respectively. Excretion was rapid, with more than 95% of the radioactivity excreted within 48 hours. Urinary excretion was 24–35%, and faecal excretion was 45–69%. In bile duct cannulated rats administered a [^{14}C]tioxazafen dose of 100 mg/kg bw, approximately 45–60% of the radiolabel was recovered in the bile, 21–45% in the urine and 3.3–11% in the faeces over 48 hours post-dosing, indicating that at least 89% of the administered dose was absorbed. There were no major differences in excretion or metabolism due to dose, sex or dosing regimen. Tissue distribution was widespread, but levels in tissues were low. Highest levels were found in adrenals, kidneys, liver and thyroid.

Tioxazafen was extensively metabolized to approximately 30 components in rats. No parent compound was found in urine, faeces or bile. Major routes of metabolism of tioxazafen in rats were oxidation (hydroxylation) of the thiophene ring, followed by conjugation primarily with glucuronic acid, and reductive cleavage and subsequent hydrolysis of the oxadiazole ring. The major metabolites were benzamidine, hydroxyl-tioxazafen glucuronide and thenoylglycine. Benzamidine was the only metabolite that was recovered in urine at more than 10% of the administered dose.

Toxicological data

The acute oral LD_{50} in rats was greater than 5000 mg/kg bw, the acute dermal LD_{50} was greater than 5000 mg/kg bw and the acute inhalation LC_{50} was greater than 5.2 mg/L. Tioxazafen was not irritating to the skin and was mildly irritating to the eyes of rabbits. Tioxazafen was not skin sensitizing in a maximization test in guinea-pigs.

In repeated-dose oral toxicity studies with tioxazafen in mice, rats and dogs, a number of effects were observed, most notably reduced body weight gain, increased liver weight, hepatocellular hypertrophy, increased levels of bilirubin and cholesterol, haematological changes, histopathological changes in the adrenals and hyperostosis (bone thickening).

In a 28-day range-finding study in mice using dietary tioxazafen concentrations of 0, 20, 100, 300, 1000 and 3000 ppm (equal to 0, 4, 19, 58, 184 and 437 mg/kg bw per day for males and 0, 5, 25, 70, 219 and 399 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 58 mg/kg bw per day), based on increased bilirubin, liver weights and hepatocellular hypertrophy in both sexes, increased

cholesterol and GGT levels in females, and termination of one female in extremis at 1000 ppm (equal to 184 mg/kg bw per day). All animals in the 3000 ppm group died or were terminated early.

In a 90-day study in mice using dietary tioxazafen concentrations of 0, 10, 50, 200, 600 and 1250 ppm (equal to 0, 2.1, 10.3, 42, 125 and 260 mg/kg bw per day for males and 0, 2.6, 13.8, 54, 174 and 319 mg/kg bw per day for females, respectively), the NOAEL was 600 ppm (equal to 125 mg/kg bw per day), based on increased bilirubin and cholesterol levels in females, increased liver weights and hepatocellular hypertrophy in both sexes, and termination of one female in extremis at 1250 ppm (equal to 260 mg/kg bw per day).

In a 28-day study in rats using dietary tioxazafen concentrations of 0, 50, 200, 1000, 3000 and 10 000 ppm (equal to 0, 4, 15, 76, 201 and 628 mg/kg bw per day for males and 0, 5, 18, 89, 221 and 760 mg/kg bw per day for females, respectively), the NOAEL was 200 ppm (equal to 15 mg/kg bw per day), based on decreases in body weight gain and histopathological changes in the liver in males and decreased feed consumption and feed efficiency, hyperostosis and increased adipose tissue of the sternal bone marrow in both sexes at 1000 ppm (equal to 76 mg/kg bw per day).

In a 90-day study in rats using dietary tioxazafen concentrations of 0, 10, 50, 250, 750 and 1500 ppm (equal to 0, 1, 3, 16, 47 and 91 mg/kg bw per day for males and 0, 1, 4, 19, 55 and 113 mg/kg bw per day for females, respectively), the NOAEL was 250 ppm (equal to 16 mg/kg bw per day), based on a reduction in body weight gain in females and hyperostosis in both sexes at 750 ppm (equal to 47 mg/kg bw per day).

In a 13-week oral toxicity study in dogs administered tioxazafen by gelatine capsule at a dose of 0, 1, 3, 10, 40 or 120 mg/kg bw per day, the NOAEL was 40 mg/kg bw per day, based on an increase in lung weights in both sexes and one female mortality at 120 mg/kg bw per day.

In an 18-month carcinogenicity study in mice using dietary concentrations of 0, 5, 50, 250, 750 (both sexes) and 1750 ppm (males only) (equal to 0, 1, 8, 41, 120 and 282 mg/kg bw per day for males and 0, 1, 10, 50 and 153 mg/kg bw per day for females, respectively), the NOAEL for systemic toxicity was 50 ppm (equal to 10 mg/kg bw per day), based on increases in pigmented macrophages with scattered necrotic hepatocytes and centrilobular hepatocellular hypertrophy in females at 250 ppm (equal to 50 mg/kg bw per day). The NOAEL for carcinogenicity was 250 ppm (equal to 50 mg/kg bw per day), based on an increased incidence of hepatocellular adenomas in females at 750 ppm (equal to 153 mg/kg bw per day). The incidence of hepatocellular carcinomas, but not of adenomas, was increased in males at 1750 ppm, and there was equivocal evidence of increases in the incidence of systemic haemangiosarcomas at 1750 ppm in males and of histiocytic sarcomas at 750 ppm in females.

Three studies were performed to investigate the mode of action for the observed tumours in the carcinogenicity study in mice. Mice were administered tioxazafen in their diet for 4–90 days at doses ranging from 20 to 1750 ppm in males and from 10 to 750 ppm in females. Tumorigenic doses of tioxazafen induced increased liver weights and serum ALT, AST, sorbitol dehydrogenase and total bilirubin levels in mice. Histopathology showed hepatocellular degeneration, centrilobular necrosis, inflammation, fatty changes, increased mitoses and histiocytic infiltration. Tioxazafen also induced marked increases in hepatocellular proliferation (5-bromo-2'-deoxyuridine labelling), in particular in the periportal region, as is commonly observed with chemically induced cytotoxicity. Observed increases in endothelial cell proliferation were considered secondary to the hepatocellular toxicity and increased hepatocellular proliferation. The effects were predominantly observed in males at 1750 ppm and, to a lesser extent, in females at 750 ppm. There was no biologically meaningful activation of the aryl hydrocarbon receptor, CAR, PXR or peroxisome proliferator-activated receptors alpha and gamma, indicating that the tumour induction was unrelated to modes of action involving activation of these nuclear hormone receptors. No effects on

two markers for angiogenesis or hypoxia were observed. Based on these results, the hepatocellular carcinogenicity observed at the high doses in the 18-month mouse feeding study was considered to be a result of a cytotoxic mode of action. This mode of action is relevant to humans, but exhibits a threshold, because tumours would not occur in the absence of hepatotoxicity.

In a 2-year carcinogenicity study in rats using dietary concentrations of 0, 5, 25, 75, 250 and 750 ppm (equal to 0, 0.3, 1.3, 3.9, 13.3 and 39.6 mg/kg bw per day for males and 0, 0.3, 1.6, 4.9, 16.0 and 48.1 mg/kg bw per day for females, respectively), the NOAEL for systemic toxicity was 75 ppm (equal to 4.9 mg/kg bw per day), based on an increased incidence of endometrial stromal polyps in females at 250 ppm (equal to 16.0 mg/kg bw per day). This lesion is a common, benign, noncancerous finding in female rodents. Although certain types of uterine polyps can progress to cancer in rare cases, there is no instance of this occurring in the absence of other indications of malignancy (i.e. evidence of preneoplastic changes in the uterus, tumours at other sites). There was no increase in any tumour type in rats that could be attributed to treatment with tioxazafen. The NOAEL for carcinogenicity was 750 ppm (equal to 39.6 mg/kg bw per day), the highest dose tested.

The Meeting concluded that tioxazafen is carcinogenic in mice, but not in rats.

Tioxazafen was tested for genotoxicity in an adequate range of in vitro and in vivo assays. No evidence of genotoxicity was found.

The Meeting concluded that tioxazafen is unlikely to be genotoxic.

In view of the lack of genotoxicity, the absence of carcinogenicity in rats, the fact that hepatocellular adenomas and carcinomas were increased in mice by a cytotoxic mode of action and the fact that there was an equivocal increase in the incidence of systemic haemangiosarcomas in male mice and of histiocytic sarcomas in female mice only at the highest dose tested, the Meeting concluded that tioxazafen is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation reproductive toxicity study in rats administered tioxazafen in the diet at a dose of 0, 5, 20 or 60 mg/kg bw per day (concentrations were adjusted weekly to provide target test substance doses), the NOAEL for parental toxicity was 20 mg/kg bw per day, based on reduced body weight gains and hyperostosis in F_0 and F_1 males at 60 mg/kg bw per day. The NOAEL for offspring toxicity was 60 mg/kg bw per day, the highest dose tested. The NOAEL for reproductive toxicity was 60 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study of tioxazafen in rats using gavage doses of 0, 10, 50 and 200 mg/kg bw per day from gestational days 6 to 19, the NOAEL for maternal toxicity was 10 mg/kg bw per day, based on reduced feed intake and body weight gain at 50 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 200 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits administered tioxazafen by gavage at a dose of 0, 5, 20 or 100 mg/kg bw per day from gestational days 7 to 28, the NOAEL for maternal toxicity was 5 mg/kg bw per day, based on reduced body weight gain at 20 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, the highest dose tested.

The Meeting concluded that tioxazafen is not teratogenic.

In an acute neurotoxicity study in which rats were administered tioxazafen by gavage at a dose of 0, 250, 750 or 2000 mg/kg bw and then observed for 14 days, no NOAEL could be identified. The LOAEL for neurotoxicity was 250 mg/kg bw, the lowest dose tested, based on a transient decrease in motor activity observed 4 hours after treatment in males and females at this dose in the absence of any neuropathological changes.

In a 13-week neurotoxicity study in rats using dietary tioxazafen concentrations of 0, 100, 300 and 1000 ppm (equal to 0, 7, 20 and 67 mg/kg bw per day for males and 0, 8, 24 and 75 mg/kg bw per day for females, respectively), the NOAEL for systemic toxicity was 100 ppm (equal to 8 mg/kg bw per day), based on decreased body weight gain in females at 300 ppm (equal to 24 mg/kg bw per day). There was no evidence of a neurotoxic effect of tioxazafen, and the NOAEL for neurotoxicity was 1000 ppm (equal to 67 mg/kg bw per day), the highest dose tested.

Although there were no indications of neuropathological effects of tioxazafen, the Meeting concluded that tioxazafen may cause transient, acute neurobehavioural effects at high doses.

In a 28-day immunotoxicity study in female mice using dietary tioxazafen concentrations of 0, 100, 300 and 1000 ppm (equal to 0, 26, 80 and 240 mg/kg bw per day, respectively), no signs of an immunotoxic effect were observed. The NOAEL for systemic toxicity was 300 ppm (equal to 80 mg/kg bw per day), based on an increase in bilirubin levels, higher absolute and relative liver weights (17–18%) and minimal to mild centrilobular hepatocellular hypertrophy at 1000 ppm (equal to 240 mg/kg bw per day).

The Meeting concluded that tioxazafen is not immunotoxic.

Toxicological data on metabolites and/or degradates

The major residues in crops and livestock were tioxazafen and benzamidine. No specific toxicity studies on benzamidine were available. However, this metabolite occurs in rat urine at up to about 12.6% of the administered dose.

The Meeting concluded that the toxicity of benzamidine would be covered by that of tioxazafen.

Toxicological data on MON 102130 (3-phenyl-5-thiophen-3-yl-1,2,4-oxadiazole), a photolyte of tioxazafen, were available. The acute oral LD_{50} of MON 102130 was greater than 5000 mg/kg bw. In a 28-day study in rats using dietary MON 102130 concentrations of 0, 200, 1000 and 3000 ppm (equal to 0, 15, 72 and 207 mg/kg bw per day for males and 0, 16, 77 and 211 mg/kg bw per day for females, respectively), the NOAEL was 200 ppm (15 mg/kg bw per day), based on decreased body weight, body weight gain and feed consumption, alterations in haematological and clinical chemistry parameters, organ weight changes and histopathological changes in the liver in both sexes at 1000 ppm (equal to 72 mg/kg bw per day). MON 102130 was negative in a bacterial reverse mutation assay and in an in vivo micronucleus test in mice.

The Meeting concluded that MON 102130 is of similar potency to tioxazafen.

Human data

Skin rashes were observed in a limited number of individuals who were potentially exposed to tioxazafen.

The Meeting concluded that the existing database on tioxazafen was adequate to characterise the potential hazards to the general population, including fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.05 mg/kg bw for tioxazafen on the basis of a NOAEL of 4.9 mg/kg bw per day in a 2-year rat study, based on a small increase in the incidence of endometrial stromal polyps in females at 16.0 mg/kg bw per day. A safety factor of 100 was used. The upper bound of the ADI gives a margin of about 3000 relative to the LOAEL for the observed tumours in mice. The ADI is supported by a NOAEL of 5 mg/kg bw per day, based on reduced maternal body weight gain observed at 20 mg/kg bw per day, in a developmental toxicity study in rabbits, and a NOAEL of 8 mg/kg bw per day, based on decreased body weight gain in females at 24 mg/kg bw per day, in a 13-week neurotoxicity study in rats.

The Meeting established an ARfD of 0.5 mg/kg bw for tioxazafen on the basis of a LOAEL of 250 mg/kg bw, based on a reduction in locomotor activity in an acute neurotoxicity study in rats. A safety factor of 500 was used. An additional factor of 5 was applied for the use of a LOAEL instead of a NOAEL. The Meeting noted that no neurobehavioural signs were observed in any of the repeated-dose studies at bolus doses up to 120 mg/kg bw per day.

The ADI and ARfD can be applied to benzamidine.

A toxicological monograph was prepared.

Levels relevant to risk assessment of tioxazafen

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of carcinogenicity[a]	Toxicity	50 ppm, equal to 10 mg/kg bw per day	250 ppm, equal to 50 mg/kg bw per day
		Carcinogenicity	250 ppm, equal to 50 mg/kg bw per day	750 ppm, equal to 153 mg/kg bw per day
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	75 ppm, equal to 4.9 mg/kg bw per day	250 ppm, equal to 16.0 mg/kg bw per day
		Carcinogenicity	750 ppm, equal to 39.6 mg/kg bw per day[b]	–
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	60 mg/kg bw per day[b]	–
		Parental toxicity	20 mg/kg bw per day	60 mg/kg bw per day
		Offspring toxicity	60 mg/kg bw per day[b]	–
	Developmental toxicity study[c]	Maternal toxicity	10 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	200 mg/kg bw per day[b]	–
	Acute neurotoxicity study[c]	Neurotoxicity	–	250 mg/kg bw[d]
	Thirteen-week neurotoxicity study[a]	Toxicity	100 ppm, equal to 8 mg/kg bw per day	300 ppm, equal to 24 mg/kg bw per day
		Neurotoxicity	1 000 ppm, equal to 67 mg/kg bw per day[b]	–
Rabbit	Developmental toxicity study[c]	Maternal toxicity	5 mg/kg bw per day	20 mg/kg bw per day
		Embryo and fetal toxicity	100 mg/kg bw per day[b]	–
Dog	Thirteen-week study of toxicity[e]	Toxicity	40 mg/kg bw per day	120 mg/kg bw per day

[a] Dietary administration.

[b] Highest dose tested.

[c] Gavage administration.

[d] Lowest dose tested.

[e] Capsule administration.

Acceptable daily intake (ADI) (applies to tioxazafen and benzamidine, expressed as tioxazafen)

0–0.05 mg/kg bw

Acute reference dose (ARfD) (applies to tioxazafen and benzamidine, expressed as tioxazafen)

0.5 mg/kg bw

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid (T_{max} 2–4 hours) and almost complete (89–97%) in rats
Dermal absorption	7.45%, 1.59% and 1.65% at 4.5, 45 and 450 g/L, respectively (in vivo, rat) 0.52–4.48% (in vitro, human) 8–32% (in vitro, rat)
Distribution	Widely distributed, highest concentrations found in adrenals, kidney, liver and thyroid
Potential for accumulation	None
Rate and extent of excretion	Rapid; 95% in 48 hours
Metabolism in animals	Extensively metabolized, major metabolites are benzamidine, hydroxyl-tioxazafen glucuronide and thenoylglycine
Toxicologically significant compounds in animals and plants	Tioxazafen
Acute toxicity	
Rat, LD_{50}, oral	>5 000 mg/kg bw
Rat, LD_{50}, dermal	>5 000 mg/kg bw
Rat, LC_{50}, inhalation	>5.2 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Mildly irritating
Guinea-pig, dermal sensitization	Sensitizing (Buehler maximization test)
Short-term studies of toxicity	
Target/critical effect	Body weight gain, liver, haematological effects, adrenals, hyperostosis
Lowest relevant oral NOAEL	15 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	100 mg/kg bw per day (rat)

Lowest relevant inhalation NOAEC	15 mg/m^3 (rat)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Uterus, liver
Lowest relevant NOAEL	4.9 mg/kg bw per day (rat)
Carcinogenicity	Carcinogenic in mice, but not in rats[a]
Genotoxicity	
	No evidence of genotoxicity[a]
Reproductive toxicity	
Target/critical effect	No reproductive effects
Lowest relevant parental NOAEL	20 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	60 mg/kg bw per day, highest dose tested (rat)
Lowest relevant reproductive NOAEL	60 mg/kg bw per day, highest dose tested (rat)
Developmental toxicity	
Target/critical effect	No developmental toxicity
Lowest relevant maternal NOAEL	5 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	100 mg/kg bw per day, highest dose tested (rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	250 mg/kg bw, lowest dose tested (rat)
Subchronic neurotoxicity NOAEL	67 mg/kg bw per day, highest dose tested (rat)
Developmental neurotoxicity NOAEL	No data
Immunotoxicity	
Twenty-eight-day immunotoxicity NOAEL	240 mg/kg bw per day, highest dose tested (mouse)
Studies on metabolites	
MON 102130 (photolyte of tioxazafen)	LD_{50} > 5 000 mg/kg bw 28-day oral toxicity NOAEL 15 mg/kg bw per day No evidence of genotoxicity
Human data	
	Skin rashes were observed in a limited number of individuals who were potentially exposed to tioxazafen

[a] Unlikely to pose a carcinogenic risk to humans via exposure from the diet.

Summary

	Value	Study	Safety factor
ADI	0–0.05 mg/kg bw[a]	Two-year toxicity study in rats	100
ARfD	0.5 mg/kg bw[a]	Acute neurotoxicity study in rats	500

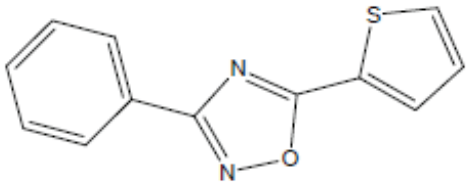
[a] Applies to tioxazafen and benzamidine, expressed as tioxazafen.

RESIDUE AND ANALYTICAL ASPECTS

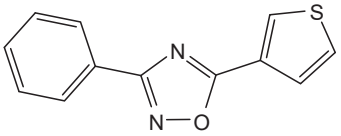
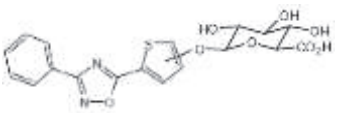
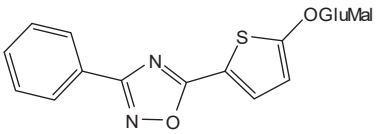
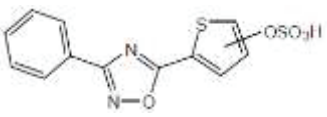
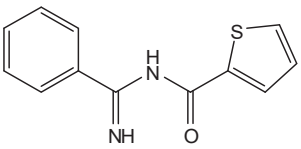
Tioxazafen is a seed treatment nematicide to control a broad-spectrum of nematodes in maize, soya bean, and cotton. Tioxazafen is a disubstituted oxadiazole, which represents a new class of nematicidal chemistry demonstrating activity against soya bean cyst, root knot and reniform nematodes in soya bean; lesion, root knot and needle nematodes in maize; as well as reniform and root knot nematodes in cotton.

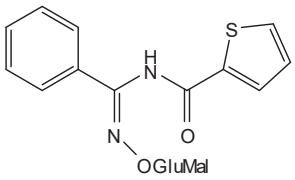
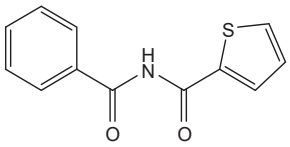
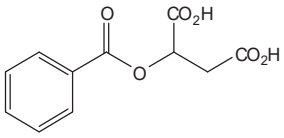
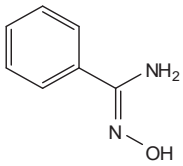
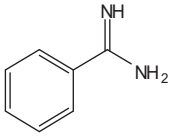
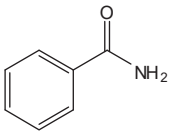
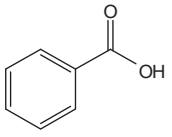
Tioxazafen was scheduled at the Forty-ninth Session of the CCPR for new evaluation, as a new ompound, for residues and toxicology by the 2018 JMPR. The meeting received information on the physical and chemical properties, metabolism in crops, rotational crop studies, metabolism in animals, environmental fate in soil and water, methods of residue analysis, stability in stored analytical samples, use patterns, supervised residue trials, fate of residue during storage and processing, and livestock feeding studies.

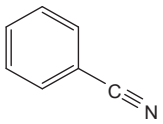
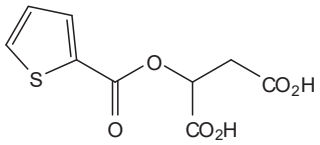
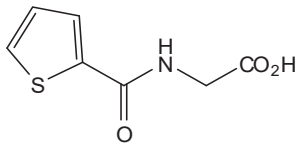
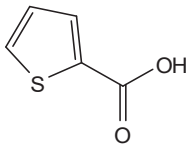
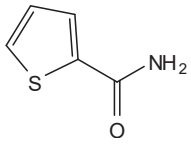
The IUPAC name of tioxazafen is 3-phenyl-5-thiophen-2-yl-1,2,4-oxadiazole



The following abbreviations are used for the metabolites discussed in the appraisal:

Trivial Name	Chemical Name	Structure	Where Found
3-Thienyl tioxazafen (MON 102130)	3-phenyl-5-(3-thienyl)-1,2,4-oxadiazole		Aqueous photolysis, soil photolysis
Hydroxy tioxazafen Glucuronide	(Isomeric position of glucuronide not confirmed in metabolite isolated from goat)		Goat skim milk, goat milk fat
Hydroxy tioxazafen Malonylglucoside	5-[5-[6-*O*-(2-carboxyacetyl)-β-D-glucopyranosyloxy]-2-thienyl]-3-phenyl-1,2,4-oxadiazole		Soya bean foliage, soya bean seed
Hydroxy tioxazafen Sulfate	(Isomeric position of sulfate not confirmed in metabolite isolated from hen and goat)		Hen egg, goat milk fat
MON tioxazafen Iminoamide	*N*-(iminophenylmethyl)-2-thiophenecarboxamide		Rotational crops anaerobic aquatic, aerobic aquatic, anaerobic soil

Trivial Name	Chemical Name	Structure	Where Found
Thenoylbenzamidoxime Malonylglucoside (hydroxy iminoamide malonyl glucoside)	*O*-[6-*O*-(2-carboxyacetyl)-β-D-glucopyranosyl]-*N*-(2-thenoyl)-benzamidoxime		Maize foliage, soya bean foliage
Tioxazafen Imide	*N*-benzoyl-2-thiophene		Rotational crops, hen liver, hen egg, hen excreta aerobic aquatic, aerobic soil, anaerobic aquatic, anaerobic soil
Benzoylmalic acid	2-(benzoyloxy)butanedioic acid		Soya bean foliage
Benzamidoxime	*N*-hydroxybenzene		Maize foliage, cotton foliage
Benzamidine	benzenecarboxamidamide		Maize foliage, soya bean foliage, soya bean seed, cotton foliage, rotational crops, hen liver, hen muscle, hen egg, hen excreta, goat liver, goat kidney, goat muscle, goat skim milk, goat fat anaerobic aquatic, aerobic aquatic, anaerobic soil
Benzamide	benzamide		Maize foliage, cotton foliage, rotational crops, hen liver, hen egg, hen excreta, goat liver, goat kidney, goat skim milk
Benzoic acid	benzoic acid		Maize foliage, cotton foliage, rotational crops; Hen excreta, goat liver, goat skim milk anaerobic soil

Trivial Name	Chemical Name	Structure	Where Found
Benzonitrile	benzonitrile		Hen fat, goat fat
Thenoylmalic acid (Thenoylmalate)	2-(2-thienylcarbonyloxy) acid		Soya bean foliage
Thenoylglycine (2-Thenoylglycine)	*N*-(2-thienylcarbonyl) glycine		Goat liver, goat kidney, goat skim milk, goat milk fat (glycine conjugate of thiophene acid)
Thiophene acid	2-thiophenecarboxylic acid		Maize foliage, cotton foliage, rotational crops; present as conjugates Hen excreta, goat liver, goat kidney (as thenoylglycine) anaerobic aquatic, aerobic aquatic, anaerobic soil
Thiophene amide	2-thiophenecarboxamide		Maize foliage, rotational crops

Tioxazafen is a compound with low solubility in water and low volatility, and a potential for bioaccumulation. Tioxazafen is hydrolytically and photolytically stable.

Tioxazafen is only registered as a seed treatment.

Studies on the metabolism in plants, livestock and environmental fate utilised either [oxadiazole-3-^{13}C, phenyl-U-^{14}C]-tioxazafen (PH-T) or [oxadiazole-5-^{13}C, thiophene-2-^{14}C]-tioxazafen (TH-T).

Environmental fate

The Meeting received studies on the degradation of tioxazafen under aerobic condition, anaerobic condition, hydrolysis and photolysis.

Tioxazafen is stable to hydrolysis in sterile aqueous buffer solutions at pH 4, 7 and 9 at 50 °C in the dark for 5 days.

Tioxazafen, applied at a rate of 1.4 kg ai/ha is photolytically stable on non-sterile Hoyleton silt loam soil surfaces (pH 7.3, 2.1% organic matter) after 15 days of sunlight exposure. The only degradate observed after 15 days irradiation was 3-thienyl tioxazafen (3.0–3.6% AR).

Tioxazafen dissipated in aerobic soil conditions at a moderate rate (DT_{50} of 51–57.1 days and DT_{90} of 169–190 days at 20 °C) in silt loam soil, while at a much slower rate in sandy clay loam and clay loam

soil with DT_{50s} of 141–144 days and 221–277 days, respectively, and DT_{90s} ranging from 524 days to >1000 days. In a field dissipation study, the DT_{50} values ranged from 15 days to 289 days with a median of 70 days and an average of 111 days in the treated seed plot. In the treated in-furrow plot, the DT_{50} values ranged from 40 to 101 days with a median of 89 days and an average of 80 days. The DT_{90} values for tioxazafen dissipation in both treated seed and in-furrow treatment plots were less than a year except in treated seed plots in Manitoba where the DT_{90} was 960 days.

The formation of bound residues and mineralization to $^{14}CO_2$ were principal routes of dissipation. The total of the unidentified components never exceeded 2.4% AR. The dissipation of tioxazafen was characterised by a rapid initial decline of approximately 10–20% over the first 3–5 days followed by a slower dissipation phase.

The pattern of tioxazafen decline showed a slowing of the dissipation rate during the fall/winter months. Dissipation of tioxazafen occurred at a moderate rate in the treated seed plots and treated in-furrow plots. There were no residues of benzamidine above 0.0015 mg/kg in 15–30cm soil, and less than 0.024 mg/kg in 0–7.5cm soil.

Therefore, the potential for significant amounts of tioxazafen to carry over into the following season is relatively low except in cold climate conditions. The carry-over of benzamidine would be insignificant.

Plant metabolism

The Meeting received plant metabolism studies with seed treatment of tioxazafen to genetically modified (GM) soya bean, GM maize and cotton.

Soya bean

GM soya bean seeds were treated with a suspension concentrate (SC) formulation of ^{14}C-tioxazafen, labelled in the phenyl ring (PH-T) or the thiophene ring (TH-T), at rates of 1.30 mg ai/seed (≈0.81 kg ai/ha) for the PH-T treatment and 1.26 mg ai/seed (≈0.78 kg ai/ha) for the TH-T treatment. Treated soya bean seeds were planted outdoors in loamy sand soil. Samples of plant thinnings (immature foliage at BBCH 12), forage (BBCH 17), hay (at mid-to-full bloom stage or pods are approximately 50% developed) and seed were collected at 28, 48, 88 and 147 days after planting, respectively.

Residue levels (expressed as parent tioxazafen equivalents) were highest in thinnings (9.0–11 mg eq/kg), decreased substantially in forage (0.43–0.51 mg eq/kg) and hay (0.78–1.1 mg eq/kg), and were lowest in seed (0.070–0.16 mg eq/kg). Extractability was moderate with 56–62% of TRR in forage and 52–56% of TRR in hay extracted with acetone and water while 70% of TRR in seeds was extracted with hexane and acetone.

Tioxazafen was extensively metabolised in thinnings, forage, hay and seed. Tioxazafen (parent) levels in thinnings were 5.6% of the TRR (0.51 mg eq/kg) for the PH-T and 4.7% (0.51 mg eq/kg) for TH-T while levels in the forage and hay were 4.3–13% of the TRR (0.026–0.054 mg eq/kg). Based on solvent partitioning properties, tioxazafen in seeds would have represented no more than 0.9% of the TRR (0.0006 mg eq/kg) for the PH-T and 0.5% of the TRR (0.0008 mg eq/kg) for the TH-T label.

Benzamidine was the only metabolite identified in seed (11% TRR, 0.0076 mg eq/kg).

Benzamidine was also the major metabolite in thinnings (11% TRR, 0.96 mg eq/kg), forage (8.5% TRR, 0.036 mg eq/kg) and hay (8.1% TRR, 0.063 mg eq/kg) from PH-T treatment. Other metabolites identified were thenoylbenzamidoxime malonylglucoside (2.4-4.1% TRR, 0.011–0.45 mg eq/kg), hydroxy (thiophene) tioxazafen malonylglucoside (0.9–4.9% TRR, 0.0067–0.54 mg eq/kg), benzoylmalic acid (8.5%

TRR, 0.77mg eq/kg, thinnings only) and thenoylmalic acid (3.6% TRR, 0.40mg eq/kg, thinnings only). An unknown metabolite with MW 365 (5% of TRR, 0.45 mg eq/kg) was characterised in PH-T thinnings, and an unknown metabolite (5.6% of TRR, 0.614 mg eq/kg) was characterised in TH-T thinnings.

Most of the radioactivity in PES in forage and hay was associated with lignin (15.7–17.8% of TRR) and hemicellulose (6.9–14.5% of TRR).

The Meeting noted that a genetically modified variety of soya bean was used in the metabolism study. However, the modification is designed to increase the tolerance to glyphosate and acetolactate synthase inhibitor herbicides, and is unlikely to impact the metabolism of tioxazafen in soya bean.

Maize

GM maize seeds were treated with an SC formulation of ^{14}C tioxazafen containing PH-T or TH-T labelled compound at rates of 1.09 mg ai/seed (≈0.26 kg ai/ha) for the PH-T treatment and 1.28 mg ai/seed (≈0.30 kg ai/ha) for the TH-T treatment. Treated seeds were planted outdoors in loamy sand soil. Samples of thinnings (immature foliage), forage, stover and grain were collected 24, 101 and 130 days after planting.

Similar to soya bean, tioxazafen is extensively metabolised in maize. Residue levels in thinnings were 1.72–1.97 mg eq/kg, forage 0.0084–0.015 mg eq/kg, stover 0.042–0.064 mg eq/kg and grain 0.0012–0.0020 mg eq/kg. Extractability in the solvent system employed (acetone/water) was 83–84% TRR for thinnings, 68–71% TRR for forage, 67–68% TRR for stover and 12–42% TRR for grain. The characterisation and identification of residues in grain was not conducted due to very low levels of radioactivity.

Parent tioxazafen was a major residual component in thinnings harvested about two weeks after emergence (33–46% TRR, 0.57–0.91 mg eq/kg), but did not exceed 1% of TRR in forage or stover.

Benzamidine was identified as the only major metabolite in thinnings (8.8% TRR, 0.15 mg eq/kg), forage (12% of TRR, 0.0018 mg eq/kg) and stover (11% of TRR, 0.0072 mg eq/kg). Other minor metabolites identified were thenoylbenzamidoxime malonyl glucoside (0.083–0.12 mg eq/kg, 4.2–7.1% TRR) in the thinnings; benzamide in thinnings (1.9% TRR, 0.033 mg eq/kg), forage (4.8% TRR, 0.0007 mg eq/kg) and stover (4.0% TRR, 0.0026 mg eq/kg); and benzoic acid and thiophene-2-carboxylic acid at trace levels. No single metabolite exceeded 0.01 mg eq/kg in maize forage or stover.

The Meeting noted that genetically modified variety of maize was used in the metabolism study. However, the modification is designed to increase the tolerance to glyphosate and acetolactate synthase inhibitor herbicides, and is unlikely to impact the metabolism of tioxazafen in maize.

Cotton

Pima cotton seeds were treated with an SC formulation of ^{14}C tioxazafen at rates of 1.20 mg ai/seed (≈0.28 kg ai/ha) for the PH-T treatment and 1.30 mg ai/seed (≈0.31 kg ai/ha) for the TH-T treatment. Treated cotton seeds were planted outdoors in loamy sand soil. Samples of thinnings, leaves/stems and undelinted seed were collected at 39 and 182 days after planting.

Radioactivity levels were highest in thinnings (1.04–2.40 mg eq/kg) followed by leaves/stems (0.063–0.065 mg eq/kg) and undelinted seed (0.0087 –0.009 mg eq/kg). Solvent extractability was 69–74% TRR for thinnings and 71–79% TRR for leaves and stems using acetone/water and 38–43% TRR for undelinted seed (recombined seed and lint) using hexane, acetone and acetone/water. Harsh treatment of PES from PH-T and TH-T thinnings with 0.1 M KOH and 24% KOH released a further 12–13% and 13–16% TRR, respectively, while for leaves/stems the treatments released a further 2.8–3.0% and 9.4–13% TRR, respectively.

Tioxazafen is extensively metabolized in cotton. Parent tioxazafen was only identified in thinnings (6.3–16% TRR, 0.065–0.38 mg eq/kg) and was not detected in leaves/stems. Due to the low levels of solvent extracted radioactivity in undelinted seed (0.004 mg eq/kg) identification was not conducted for this matrix. Based on solvent partitioning properties, if tioxazafen was present in undelinted seed accounted for no more than 1% TRR (< 0.001 mg eq/kg). No single metabolite exceeded 0.01 mg eq/kg in leaves/stems.

Benzamidine was identified as a major metabolite in thinnings (6.2% TRR, 0.064 mg eq/kg) and leaves/stems (11% TRR, 0.0071 mg eq/kg). Thiophene-2-carboxylic acid was identified as a major metabolite in thinnings (9.4% TRR, 0.226 mg eq/kg) and leaves/stem (7.9% TRR, 0.005 mg eq/kg). Other minor metabolites identified were benzamide in thinnings (4.0% TRR, 0.042 mg eq/kg) and leaves/stems (7.6% TRR, 0.005 mg eq/kg); benzoic acid in thinnings (7.5% TRR, 0.078 mg eq/kg) and in leaves and stem (3.7% TRR, 0.0024 mg eq/kg); and 2-thiophenecarboxamide only in thinnings (0.8% TRR, 0.019 mg eq/kg). Low levels of benzoic acid and thiophene-2-carboxylic acid were presented as conjugates.

In summary, the metabolism of tioxazafen following seed treatment of the crops investigated is well understood consisting of cleavage of the oxadiazole ring and/or oxidation of the thiophene ring of tioxazafen, followed by hydrolysis of benzamidine and its conjugation. In addition, hydroxylation of the phenyl ring of tioxazafen was also observed. The concentration of parent tioxazafen was too low to be identified in seed and grain. The predominant metabolite identified is benzamidine, which is also found in the rat study.

Rotational crop studies

The meeting received a confined rotational crop study and a field rotational crop study.

Confined rotational crop studies

Maize seeds treated with phenyl or thiophene-^{14}C labelled tioxazafen at a rate of 0.5 mg ai/seed (≈0.320 kg ai/ha based on 64 seeds in 1 m^2) were planted in sandy loam soil. The maize seedlings were cut off near the soil surface at approximately two weeks after emergence, and were chopped and tilled into the soil 30 days prior to planting of the rotational crop. Leaf lettuce, radish and wheat were grown in the soil after intervals of 30, 120 and 360 days (413 days for lettuce) as rotational crops. Immature lettuce was harvested at approximately half-size compared to commercial harvest with all remaining lettuce harvested at maturity. The tops and roots of radishes were harvested at maturity. The wheat forage was sampled prior to boot stage, the wheat hay was harvested at early flower to soft dough stage and the remaining wheat was harvested at maturity.

The TRRs in lettuce planted at 30, 120 and 413 days were all less than 0.01 mg eq/kg, with the highest TRR of 0.0095 mg eq/kg in 120-day immature lettuce from the TH treatment. The TRRs in radish foliage reached maximum values of 0.010 and 0.018 mg eq/kg in the 120-day PH and TH samples, respectively, while TRRs from the 30-day and 360-day plantings were <0.006 mg eq/kg. TRRs in radish roots reached the highest values of 0.050 and 0.057 mg eq/kg for the PH and TH samples, respectively, in the 120-day planting. TRRs in radish roots were <0.015 mg eq/kg for the 30-day and 360-day plantings. The TRRs in wheat grain were all less than 0.01 mg eq/kg with the maximum of 0.007 mg eq/kg in the 120-day PH grain. The TRRs from the 120-day planting were the highest, in the wheat forage, hay and straw. TRRs in wheat straw were higher than that in forage or hay, and were 0.077 and 0.087 mg eq/kg for PH-T and TH–T, respectively, in the 120-day planting.

The residues of parent tioxazafen were less than 0.001 mg eq/kg in lettuce, radish and wheat, generally well below 1% TRR, except in the immature lettuce from the 120-day PH plot (4.4% TRR,

0.0003 mg eq/kg) and the wheat forage from the 120-day planting in the PH plot (2.5% TRR, 0.0011 mg eq/kg). Benzamidine, benzoic acid, benzamide and 2-thiophenecarboxylic acid were identified as the primary metabolites but at levels less than 0.01 mg eq/kg in lettuce, radish and wheat. One metabolite above 10% TRR in wheat forage, hay and straw for both PH and TH reached a maximum of 0.008 mg eq/kg, a level too low to permit identification.

The Meeting noted that the actual PBI for rotational crops is about 20 days more, and the residues of tioxazafen and benzamidine in rotational crops were at very low levels. The Meeting concluded that no potential residues of tioxazafen and benzamidine are expected in rotational crops.

A field rotational crop study with lettuce, radish, sorghum and wheat confirmed the conclusions from the confined rotational crop study. Tioxaxafen and benzamidine are not expected to be detected in rotational crops

Animal metabolism

Metabolism in rats was evaluated by the WHO Core Assessment Group.

Lactating goat

Two lactating goats were dosed daily for five days with either the PH or TH-labelled tioxazafen at a rate of 11 ppm feed. Milk, urine and faeces were collected twice daily in the morning before dosing and in the evening. The goats were sacrificed approximately 18–19 hours after administration of the last dose.

The total recovery of radiolabel was 87–91% with most of the administered dose recovered in faces (64% for PH and 33% for TH) and urine (20% for PH and 50% for TH).

The TRRs were highest for liver (0.33–1.1 mg eq/kg), kidney (0.22–0.38 mg eq/kg) and milk fat (maximum 0.26–0.28 mg eq/kg). The TRRs were lower for skim milk (maximum 0.032–0.083 mg eq/kg). Milk residues reached a plateau after the second dose. The TRRs for the PH label in muscle and fats were 0.052–0.055 and 0.014–0.018 mg eq/kg, respectively while the TRRs for the TH-label were < 0.001 mg eq/kg for both muscle and fat.

Solvent extractabilities with the respective solvent systems were 16–30% TRR for liver (acetonitrile/water), 36–59% for kidney (acetonitrile/water), 99–100% for muscle (acetonitrile/water and acetonitrile), 50–63% for fat (hexane/acetone followed by partitioning with acetonitrile), 92–94% for skim milk (acetonitrile/water) and 73–88% for milk fat (acetone/hexane).

Tioxazafen is extensively metabolised in lactating goats. Parent tioxazafen was not found in milk or tissues, except fat, where it was present only at low levels (9–11% TRR, 0.001–0.002 mg eq/kg).

In milk fat, a major metabolite was the sulfate conjugate of hydroxylated tioxazafen (56–68% TRR, 0.15–0.17 mg eq/kg) for both labels. The glucuronic acid conjugate of hydroxylated tioxazafen (3.9% TRR, 0.01 mg eq/kg) was a major metabolite for the PH-label. Thenoylglycine (13% TRR, 0.034 mg eq/kg) was a major component for the TH label.

In skim milk, benzamidine (29% TRR, 0.007 mg eq/kg) and the glucuronic acid conjugate of hydroxylated tioxazafen (19% TRR, 0.005 mg eq/kg) were the major metabolites for the PH-label. Thenoylglycine (65% TRR, 0.054 mg eq/kg) was the major component with the TH label. Other metabolites were found at low levels (< 10% TRR, < 0.01 mg eq/kg).

The primary metabolite from the PH-label in all tissues was free benzamidine with residues in liver 25% TRR and 0.27 mg eq/kg, kidney 44% TRR and 0.17 mg eq/kg, muscle 99% TRR and 0.052–0.054 mg eq/kg, fat 26–56% TRR and 0.006–0.008 mg eq/kg. Another 5.3% TRR (0.058 mg eq/kg) and

8.7% TRR (0.033 mg eq/kg) of benzamidine was released after harsh treatment of liver and kidney PES, respectively. Other minor metabolites found in liver and kidney were benzoic acid conjugates (liver 12% TRR, 0.13 mg eq/kg and kidney 7.0% TRR, 0.027 mg eq/kg) and benzamide (free 0.6–4.9% TRR, 0.007–0.019 mg eq/kg and conjugated 1.5–3.2% TRR, 0.010–0.035 mg eq/kg). Other metabolites were found at low levels (< 10% TRR, < 0.01 mg eq/kg).

The primary metabolite from the TH-label in kidney was thenoylglycine (22% TRR and 0.047 mg eq/kg). Other metabolites were found at low levels (< 10% TRR, < 0.01 mg eq/kg).

Laying hens

Laying hens were administered daily doses of either phenyl-^{14}C- or thiophene-^{14}C-tioxazafen via capsule for seven days at a level equivalent to 10 ppm feed. Hens were sacrificed approximately 19–21.5 hours after the last dose was administered.

The total recovery of the radioactivity was 90–91% of the administered dose. Most of the administered dose was recovered in excreta (88%). TRR in eggs reached plateau at day 6 with the highest residue of 0.18 mg eq/kg on day 7. Radioactive residues in tissues were 0.61–0.66 mg eq/kg in liver, 0.039–0.045 mg eq/kg in fat and 0.009–0.015 mg eq/kg in muscle.

Solvent extractabilities with the respective solvent systems were 17–20% TRR for liver, 34–48% for muscle, and 22–27% TRR for egg (acetonitrile/water 1:1, v/v, 2× and acetonitrile 1×), and 88–92% for fat (acetone/hexane 1:4, v/v, 1× and acetone 2×).

Tioxazafen is extensively metabolised in laying hens. Parent tioxazafen was found at trace levels in eggs or tissues, except fat, where it was the major compound (49–76% TRR, 0.021–0.034 mg eq/kg).

The major compound from the PH-label in liver, muscle and eggs was benzamidine (3.4–18% TRR, 0.002–0.035 mg eq/kg), while benzonitrile (21–30% TRR, 0.010–0.013 mg eq/kg) was the major metabolite in fat. Other metabolites were found at low levels (<10% TRR, < 0.01 mg eq/kg).

The primary metabolite from the TH-label was an unknown compound M4, likely a mixture of polar metabolites, at 12–13% TRR (0.001–0.002 mg eq/kg) in muscle and 3.5% TRR (0.023 mg eq/kg) in liver. Other metabolites were found at low levels (<10% TRR, < 0.01 mg eq/kg).

In summary, the metabolism of tioxazafen by livestock is well understood consisting of cleavage of the oxadiazole ring and/or oxidation of the thiophene ring of tioxazafen, followed by hydrolysis. In addition, hydroxylation of the phenyl ring or thiophene ring of tioxazafen was also observed. The predominant metabolite identified were benzamidine and benzonitrile in hen fat; sulfate conjugate of hydroxylated tioxazafen in milk fat; and thenoylglycine in goat liver, kidney and skim milk.

Methods of analysis

Analytical methods have been developed and validated for the determination of tioxazafen and major metabolites in plant and animal commodities. Data generation methods involved extraction with acetonitrile:water and analysis by GC-MS/MS or LC-MS/MS for tioxazafen and benzamidine in plant matrices. The LOQs for tioxazafen and benzamidine (as tioxazafen equivalents) were 0.0025–0.01 mg/kg in all matrices.

A comparison of the extractability of residues in soya bean hay, maize stover and soya bean seed by acetone/water versus acetonitrile/water extraction showed that ^{14}C-tioxazafen and ^{14}C-benzamidine could be recovered equally well with the solvent system used in metabolism studies (acetone/water) and in the analytical method (acetonitrile/water).

An enforcement method was also validated for tioxazafen and benzamidine in plant matrices. The method involves the extraction of homogenised raw agricultural commodities with 65% acetonitrile in water, with benzamidine analysis by LC-MS/MS and tioxazafen analysis by GC-MS/MS. The limit of quantitation (LOQ) is 0.0050 mg/kg for each analyte , tioxazafen and benzamidine (in tioxazafen equivalents) for plant matrices.

An analytical method was developed and validated for the determination of tioxazafen and its major metabolites, benzamidine, benzonitrile and 2- thenoylglycine in animal matrices, including milk, fat, liver, kidney, muscle and egg. The analytical method involves the extraction of fat with acetonitrile:hexane and other animal matrices with acetonitrile, analysis of tioxazafen and benzonitrile by GC-MS/MS, and analysis of benzamidine and/or 2-thenoylglycine by LC-MS/MS. The LOQs for tioxazafen and benzamidine (as tioxazafen equivalents) were 0.010 mg/kg in all matrices. The LOQ for benzonitrile (as tioxazafen equivalents) was 0.025 mg/kg in fat. The LOQs for 2-thenoylglycine (as tioxazafen equivalents) were 0.010 mg/kg for milk, and 0.025 mg/kg for kidney. The LOQ for 2-thenoylglycine (as tioxazafen equivalents) in liver was 0.06 mg/kg.

Stability of residues in stored analytical sample

The Meeting received information on the stability of tioxazafen and its major metabolites in various matrices during freezer storage (-20 °C).

Tioxazafen or benzamidine in homogenised maize grain, lettuce leaves, radish root, soya bean seed , lentil seed and whole orange fruit are stable at < -20 °C for at least nine months, which covered the storage duration in the crop metabolism studies, residue trials and processing studies. Tioxazafen, benzamidine, 2-thenoylglycine and benzonitrile are stable in animal matrices (milk, kidney, fat from cattle, liver, muscle and eggs) at -18 °C for at least six months, which covered the storage duration in the livestock metabolism and feeding studies.

Definition of the residue

The nature of the tioxazafen residues was investigated in soya bean, maize and cotton after seed treatment, and in livestock following oral administration of the test substance.

Tioxazafen was extensively metabolised to a number of metabolites and their conjugates in soya bean, maize and cotton. The ^{14}C residues in edible parts such as soya bean seed, maize grain and cotton seed were much lower than that in feed commodities such as thinning, forage, hay and stover. Parent tioxazafen was not detected in samples of seed. Tioxazafen was detected in forage and fodder commodities investigated with highest levels in maize thinnings (46% TRR).

Benzamidine (6.2–12.4%) was identified as the major metabolite in soya bean, maize and cotton forage and fodder. Other minor metabolites identified were less than 5% TRR or not consistently identified in different commodities. Therefore, none of them are suitable as a marker compound

Confined and field rotational crop studies showed that no potential residues of tioxazafen and its metabolites are expected in rotational crops. Suitable analytical methods are available to analyse the parent compound and benzamidine.

The toxicity of benzamidine is considered to be covered by that of tioxazafen. Information available to the Meeting indicated that benzamidine is not naturally occurring. The Meeting considered that the residue definition for compliance with the MRL for plant commodities should be tioxazafen and benzamidine.

In deciding which additional compounds should be included in the residue definition for risk assessment, the Meeting considered the likely occurrence of the compounds in human foods. Levels of radioactivity in grain were too low for identification. Tioxazafen and metabolites, if present are at extremely low levels.

The Meeting decided that the residue definition for dietary risk assessment for plant commodities should be the sum of tioxazafen and benzamidine.

In the lactating goat metabolism study, tioxazafen is extensively metabolised. Parent tioxazafen was not found in milk or tissues except in fat, where it was present only at low levels (9–11% TRR, 0.001–0.002 mg eq/kg). The primary metabolite in skim milk, liver, kidney, and most other tissues were benzamidine (25–99% TRR, 0.007–0.27 mg eq/kg) and thenoylglycine (1.5–65% TRR, 0.005–0.054 mg eq/kg) and glucuronic acid conjugate(18.7% TRR, 0.005 mg eq/kg). The sulfate conjugate of hydroxy tioxazafen (56–68% TRR, 0.15–0.17 mg eq/kg) is found as the major metabolite in milk fat. Minor metabolites found include benzoic acid, benzamide, 2-thiophenecarboxylic acid (1.1–1.5% TRR, 0.003–0.004 mg eq/kg in liver) and benzonitrile (1.9–8.3% TRR, 0.001 mg eq/kg).

In the laying hen metabolism study, parent tioxazafen was the major component in fat (estimated as high as 49.3–75.7% TRR, 0.021–0.034 mg eq/kg), and was found at low level in liver (0.3–0.5% TRR, 0.002–0.003 mg eq/kg) and muscle (3.7–5.6% TRR, 0.0003–0.001 mg eq/kg).

Benzamidine was found as the predominant metabolites in muscle (17.5–18.1% TRR, 0.002 mg eq/kg), in liver(6% TRR, 0.035 mg eq/kg, the only significant metabolite), and in egg (3.4–4.0% TRR, 0.003–0.006 mg eq/kg) . Benzonitrile was the major metabolite in fat (21.2–30.1% TRR, 0.010–0.013 mg eq/kg). M4, likely a mixture of polar metabolites, was the major metabolite in muscle (TH label only, 11.8–13.2% TRR, 0.001–0.002 mg eq/kg) and liver (3.5% TRR, 0.023 mg eq/kg). Minor metabolites found include benzamide, benzoic acid, benzonitrile, glucuronic acid conjugate) and sulfate conjugate.

In goats, the sulfate conjugate of hydroxyl-tioxazafen was the predominant residue in milk fat (up to 68% TRR, 0.17 mg eq/kg), following administration of 11 ppm of the parent in the diet. The Meeting noted that the maximum estimated dietary burden (0.19 ppm) is approximately 60 times lower than the dose administered in the metabolism study and milk fat represents only 4% of whole milk. The Meeting concluded that no significant levels of the sulfate conjugate of hydroxyl-tioxazafen have to be expected in milk.

Additionally, in the cattle feeding study 2-thenoylglycine was only quantified in kidneys for the 3 ppm (up to 0.048 mg/kg) and 12 ppm group (up to 0.12 mg/kg), but was not found at dose levels of 0.12 and 0.6 ppm in kidneys or in any other matrix (up to 12 ppm). The Meeting concluded that due to its singular occurrence in kidney and the low levels anticipated at the actual dietary burden, no significant residues (≥ 0.01 mg/kg) are expected for this compound.

In laying hens, benzonitrile was found in major proportions in the fat (up to 30% TRR and up to 0.013 mg eq/kg). The Meeting noted that the laying hens metabolism study conducted at 21 ppm is overdosed by a factor of more than 700 compared to the estimated poultry dietary burden and concluded that no residues of benzonitrile have to be expected in poultry matrices.

The analytical method was developed for tioxazafen, benzamidine, benzonitrile and 2-thenoylglycine in animal matrices, including milk, fat, liver, kidney, muscle and egg.

The Meeting considered the residue definition for compliance with the MRL and dietary risk assessment for animal commodities was the sum of tioxazafen and benzamidine, expressed as tioxazafen.

In summary, based on the above, the Meeting recommended the following residue definitions.

Definition of the residue for compliance with the MRL and dietary risk assessment for plant commodities: *sum of tioxazafen and benzamidine, expressed as tioxazafen*.

Definition of the residue for compliance with the MRL and dietary risk assessment for animal commodities: *sum of tioxazafen and benzamidine, expressed as tioxazafen*.

The ratio of residues in muscle to fat in the laying hen metabolism study and the cow feeding study were 0.4–0.9 fold. Therefore, the Meeting considered the residues not fat-soluble.

Results of supervised residue trials on crops

Supervised residue trial data were available for tioxazafen on maize, soya bean and cotton. The residues are reported separately for tioxazafen and benzamidine. For estimation of maximum residue level, HR and STMR, the sum of tioxazafen and benzamidine (expressed as tioxazafen) is needed. When residues were below LOQ in a commodity, the sum of LOQs is applied. The method for calculation of the total residues (the sum of tioxazafen and benzamidine) is illustrated as follows:

Tioxazafen, mg/kg	Benzamidine, mg/kg (expressed as tioxazafen)	Total, mg/kg
< 0.0025	< 0.0025	< 0.005
0.005	< 0.0025	0.0075
0.01	0.005	0.015

Maize

The critical GAP for maize in the USA is a seed treatment at rate of 1.0 mg ai/seed (maximum seasonal rate of 99 g ai/ha). The Meeting received supervised residue trial data for tioxazafen on maize conducted in the USA.

In 22 trials conducted at rates approximating critical GAP or at rates double the critical GAP, in the USA, the total residues of tioxazafen and benzamidine in maize grain (in both scenarios) were (n = 22): < 0.005 mg/kg.

The Meeting noted that the LOQs of the analytical method for enforcement are 0.005 mg/kg for each analyte, and combined the LOQs for tioxazafen and benzamidine to estimate a maximum residue level of 0.01(*) mg/kg, and a STMR of 0 mg/kg for maize grain.

Soya bean seed (dry)

The critical GAP for soya bean in the USA is a seed treatment at a rate of 0.5 mg ai/seed (maximum seasonal rate of 309 g ai/ha). The Meeting received supervised residue trial data for tioxazafen on soya bean conducted in the USA.

In trials conducted at rates approximating critical GAP in the USA, the total residues of tioxazafen and benzamidine in soya bean seed were (n = 22): 0.0055, 0.0062, 0.0063, 0.0074(2), 0.0080, 0.0092, 0.0094, 0.0096, 0.0099, 0.012, 0.013(2), 0.015(2), 0.016(2), 0.017, 0.018, 0.020, 0.022 and 0.031 mg/kg.

The Meeting estimated a maximum residue level of 0.04 mg/kg and a STMR of 0.0125 mg/kg for soya bean (dry).

Cottonseed

The critical GAP for cotton in the USA is a seed treatment at a rate of 1.0 mg ai/seed (maximum seasonal

rate of 210 g ai/ha). The Meeting received supervised residue trial data for tioxazafen on cotton conducted in the USA.

In trials conducted at rates approximating critical GAP or at rates double critical GAP in the USA, the total residues of tioxazafen and benzamidine in cotton seed (in both scenarios) were (n = 12): < 0.005 mg/kg.

The Meeting noted that the LOQs of the analytical method for enforcement are 0.005 mg/kg for each analyte, and combined the LOQs for tioxazafen and benzamidine to estimate a maximum residue level of 0.01(*) mg/kg and a STMR of 0 mg/kg for cottonseed.

Animal feed items

Maize forage and stover

The critical GAP for maize in the USA is a seed treatment at rate of 1.0 mg ai/seed (maximum seasonal rate of 99 g ai/ha). The Meeting received supervised residue trial data for tioxazafen on maize conducted in the USA.

In trials conducted at rates approximating critical GAP in the USA, the total residues of tioxazafen and benzamidine in maize stover were (n = 22): < 0.005 (13), 0.0052, 0.0056, 0.006, 0.0061, 0.007, 0.0086, 0.0087, 0.0098 and 0.013 mg/kg.

The total residue of tioxazafen and benzamidine in maize forage were (n = 22): < 0.005 (17), 0.005, 0.0055, 0.0053, 0.0054 and 0.0081 mg/kg.

The Meeting estimates a maximum residue level of 0.03 mg/kg (DM) and median residue of 0.005 mg/kg (as received) and a high residue of 0.013 mg/kg for maize stover (as received).

The Meeting estimated a median residue of 0.005 mg/kg and a high residue of 0.0081 mg/kg for maize forage (as received).

Soya bean forage and hay

The critical GAP for soya bean in the USA is a seed treatment at a rate of 0.5 mg ai/seed (maximum seasonal rate of 309 g ai/ha). The Meeting received supervised residue trial data for tioxazafen on soya bean conducted in the USA.

In trials conducted at rates approximating critical GAP in the USA, the total residues of tioxazafen and benzamidine in soya bean hay were (n = 22): 0.010, 0.026, 0.031(2), 0.032, 0.038, 0.043, 0.044, 0.045, 0.059, 0.068, 0.07, 0.077, 0.079, 0.082, 0.087, 0.095, 0.10, 0.11, 0.12(2) and 0.17 mg/kg.

The total residue of tioxazafen and benzamidine in soya bean forage were (n = 22): 0.0088, 0.0099, 0.011(2), 0.013, 0.016, 0.021, 0.023, 0.025, 0.028, 0.031, 0.034, 0.035, 0.038(2), 0.040, 0.044(2), 0.045, 0.066, 0.074 and 0.078 mg/kg.

The Meeting estimated a maximum residue level of 0.4 mg/kg (DM), a median residue of 0.069 mg/kg (as received) and a high residue of 0.17 mg/kg for soya bean hay (as received).

The Meeting estimated a median residue of 0.029mg/kg and a high residue of 0.078 mg/kg for soya bean forage (as received).

Cotton gin by products

The critical GAP for cotton in the USA is a seed treatment at rate of 1.0 mg ai/seed (maximum seasonal rate of 210 g ai/ha). The Meeting received supervised residue trial data for tioxazafen on cotton conducted

in the USA.

In trials conducted at rates approximating critical GAP in the USA, the total residues of tioxazafen and benzamidine in gin by-products were (n = 4): 0.0052, 0.0062, 0.0065 and 0.0098 mg/kg.

The Meeting estimated a maximum residue level of 0.02 mg/kg, a median residue of 0.00635 mg/kg and a high residue of 0.0098 mg/kg for gin by-product (as received).

Fate of residues during processing

The Meeting received processing studies on soya bean. A summary of the processing factors is provided below.

Commodity	Processed Fraction	Processing Factor	Best estimate PF	RAC STMR or STMR-P
Soya bean	Seeds (RAC)			0.012
	Meal	1.33, 1.49	1.41	0.017
	Hulls	0.12, 0.70	0.41	0.0049
	Refined oil	< 0.06, < 0.09	< 0 .06	0

The residues of tioxazafen concentrated in soya bean meal and meal (toasted), the Meeting estimated a maximum residue level of 0.06 mg/kg (0.04 mg/kg × 1.41) for soya bean meal.

Residues in animal commodities

Farm animal feeding studies

The Meeting received feeding studies on lactating dairy cows and laying hens.

Lactating dairy cow study

The residue levels in tissues and milk of lactating dairy cows dosed with tioxazafen at the equivalent of 0.12, 0.60, 3.0 and 12 ppm in the feed for 28 consecutive days are summarised in the following table.

Matrix	Analyte	Residues by Feeding Level[a] (mg/kg)			
		0.12 ppm (1×)	0.60 ppm (5×)	3.00 ppm (25×)	12.0 ppm (100×)
Milk	Tioxazafen	ND[b]	ND	ND	< 0.010[c]
	Benzamidine	< 0.010	< 0.010	0.0266 (0.0234)	0.0801 (0.0676)
Liver	Tioxazafen	ND	ND	ND	< 0.010
	Benzamidine	< 0.010	0.0185 (0.0143)	0.0541 (0.0473)	0.163 (0.131)
Kidney	Tioxazafen	ND	ND	ND	< 0.010
	Benzamidine	< 0.010	0.0177 (0.0150)	0.0688 (0.0650)	0.194 (0.174)
Muscle	Tioxazafen	ND	ND	ND	< 0.010
	Benzamidine	< 0.010	< 0.010	0.0141 (0.0118)	0.0410 (0.0329)
Fat[d]	Tioxazafen	ND	ND	ND	< 0.010
	Benzamidine	< 0.010	0.0114 (< 0.010)	0.0179 (< 0.010)	0.0495 (0.0211)

[a] Maximum daily average for milk. Maximum individual value for all other tissues, with dose group average in parentheses. The residues of benzamidine are reported as tioxazafen equivalents.

[b] ND = Not determined (samples not analysed because samples in higher dose group had residues less than the limit of

quantitation [LOQ])

[c] Values below the LOQ are listed as '< 0.0xx', where 0.0xx is the LOQ value for that matrix.

[d] Highest maximum value of either subcutaneous, mesenterial, or perirenal fat; average across all fat.

Laying hens study

The residue levels in tissues and eggs of laying hens dosed with tioxazafen at the equivalent of 0.81, 4.0, 21 and 79 ppm in the feed for 28 consecutive days are summarised in the following table.

Matrix	Analyte	Residues by Feeding Level (mg/kg)[a]			
		0.81 ppm (1×)	4.0 ppm (5×)	20.8 ppm (25×)	79.1 ppm (100×)
Egg	Tioxazafen	ND[b]	ND	< 0.010	0.0120 (0.0239)
	Benzamidine	ND	< 0.010	< 0.010[c]	0.0162 (0.0273)
Liver	Tioxazafen	< 0.010	< 0.010	< 0.010	< 0.010
	Benzamidine	< 0.010	0.0135 (0.0148)	0.0714 (0.0787)	0.807 (1.03)
Muscle	Tioxazafen	ND	ND	ND	< 0.010
	Benzamidine	< 0.010	< 0.010	< 0.010	0.0176 (0.0177)
Fat	Tioxazafen	< 0.010	< 0.010 (0.0106)	0.0442 (0.0519)	0.325 (0.362)
	Benzamidine	ND	ND	ND	< 0.010

[a] The overall average is listed for egg, with the maximum daily average in parentheses. The overall averages are listed for all other tissues, with the maximum individual value in parentheses. Values below the LOQ are listed as '< 0.0xx', where 0.0xx is the LOQ value for that matrix; Residues of benzamidine and benzonitrile are reported as tioxazafen equivalents.

[b] ND = Not determined. Samples not analysed because samples in the higher dose group had residues <LOQ

[c] All daily average values for benzamidine in the 25 group were < 0.010 mg/kg, but one subgroup had residues of 0.0111 mg/kg on Day 19.

Estimation of livestock dietary burdens

Dietary burdens were calculated for beef cattle, dairy cattle, broilers and laying poultry based on feed items evaluated by the JMPR. Potential cattle feed items include: maize grain, forage and stover; soya bean seed, meal, hull, forage and hay; and cotton gin by products. The dietary burdens, estimated using the OECD diets listed in Appendix IX of the 2016 edition of the FAO manual, are presented in Annex 6 and summarised below

Summary of livestock dietary burden (ppm tioxazafen equivalents of dry matter diet)

	US-Canada		EU		Australia		Japan	
	Max	Mean	Max	mean	max	Mean	max	Mean
Beef cattle	0.006	0.0044	0.02	0.014	0.19[A]	0.077[C]	0.014	0.014
Dairy cattle	0.052	0.025	0.018	0.014	0.092[B]	0.041[D]	0.019	0.016
Broilers	0.007	0.007	0.01	0.01	0.007	0.007	0.006	0.006
Layers	0.007	0.007	0.027[E]	0.015[F]	0.007	0.007	0.006	0.006

[A] Highest maximum beef or dairy cattle dietary burden suitable for maximum residue level estimates for mammalian meat

[B] Highest maximum dairy cattle dietary burden suitable for maximum residue level estimates for mammalian milk

[C] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat.

[D] Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.

[E] Highest maximum poultry dietary burden suitable for maximum residue level estimates for poultry meat and eggs

[F] Highest mean poultry dietary burden suitable for STMR estimates for poultry meat and eggs

Animal commodity maximum residue levels

The calculations used to estimate maximum residue levels, STMR and HR values for cattle matrices are shown below.

	Feed level ppm) for milk residues	Residues (mg/kg) in milk	Feed level (ppm) for tissue residues	Residues of benzamidine (mg/kg)*			
				Muscle	liver	Kidney	Fat
maximum residue level (mg/kg), beef or dairy cattle							
Feeding study	0.12	< 0.01	0.12	< 0.01	< 0.01	< 0.01	< 0.01
	0.6	< 0.01	0.6	< 0.01	0.0185	0.017	0.0114
Dietary burden and high residue estimation	0.092	< 0.01	0.19	< 0.01	0.0133	0.0128	0.01055
STMR (mg/kg), beef or dairy cattle							
Feeding study	0.12	< 0.01	0.12	< 0.01	< 0.01	< 0.01	< 0.01
Dietary burden and median residue estimated	0.041	< 0.01	0.077	< 0.01	< 0.01	< 0.01	< 0.01

*LOQs for tioxazafen and benzamidine are 0.01mg/kg; residues of tioxazafen are less than LOQ. Calculation for cattle is based on residues of benzamidine.

The maximum dietary burden calculated for cattle is 0.19 ppm for beef cattle and 0.092 ppm for dairy cattle. The mean dietary burden calculated is 0.077 ppm for beef cattle and 0.041 ppm for dairy cattle.

To estimate maximum residue levels, the LOQ for tioxazafen (0.01 mg/kg) is added to estimated benzamidine levels. The Meeting estimated a maximum residue level of 0.02 mg/kg for milk, and meat from mammals other than marine mammals; and 0.03 mg/kg (0.015 + 0.01 to nearest "step) for edible offal (mammalian) and mammalian fat. The Meeting estimated a STMR of 0.01 mg/kg for milk, meat from mammals other than marine mammals, edible offal (mammalian), and mammalian fat. The Meeting estimated a HR of 0.02 mg/kg for meat from mammals other than marine mammals, and 0.025 mg/kg for both edible offal (mammalian) and mammalian fat.

The maximum and mean dietary burden calculated for poultry (layer) are 0.027 ppm and 0.015 ppm, respectively, much lower than the lowest dose level (0.81ppm) in the feeding study, which results in residues below LOQs.

The Meeting estimated maximum residue levels of 0.02(*) mg/kg for tioxazafen in egg, poultry meat, poultry fat and poultry edible offal. The Meeting estimated STMRs of 0 mg/kg for eggs, 0.01 mg/kg for poultry meat and fat, and 0.02 mg/kg for poultry edible offal. The Meeting estimated HRs of 0.02 mg/kg for egg, poultry meat, poultry fat and poultry edible offal.

RECOMMENDATIONS

On the basis of the data obtained from supervised field trials, the Meeting concluded that the residue levels listed in Annex 1 are suitable for establishing maximum residue levels and for IEDI and IESTI assessments.

The Meeting recommended the following residue definitions for tioxazafen.

Definition of the residue for compliance with the MRL and dietary risk assessment for plant and animal commodities: *sum of tioxazafen and benzamidine (benzenecarboximidamide), expressed as tioxazafen.*

The residue is not fat-soluble.

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The ADI for tioxazafen is 0–0.05 mg/kg bw. The International Estimated Daily Intakes (IEDIs) for tioxazafen were estimated for the 17 GEMS/Food Consumption Cluster Diets using the STMR or STMR-P values estimated by the JMPR. The results are shown in Annex 3 of the 2018 JMPR Report. The IEDIs ranged from 0% of the maximum ADI.

The Meeting concluded that long-term dietary exposure to residues of tioxazafen from uses considered by the JMPR is unlikely to present a public health concern.

Acute dietary exposure

The ARfD for tioxazafen is 0.5 mg/kg bw. The International Estimate of Short Term Intakes (IESTIs) for tioxazafen were calculated for the food commodities and their processed commodities for which HRs/HR-Ps or STMRs/STMR-Ps were estimated by the present Meeting and for which consumption data were available. The results are shown in Annex 4 of the 2018 JMPR Report. The IESTIs were 0% of the ARfD.

The Meeting concluded that acute dietary exposure to residues of tioxazafen from uses considered by the present Meeting is unlikely to present a public health concern.