Tioxazafen (311)

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EXPLANATION

Tioxazafen is a seed treatment nematicide designed to provide consistent broad-spectrum control 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 evaluation as a new compound, for residues and toxicology by the 2018 JMPR. The meeting received information on 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 residues during storage and processing, and livestock feeding studies.

IDENTITY

ISO common:	Tioxazafen
Chemical name:	
IUPAC:	3-Phenyl-5-thiophen-2-yl-1,2,4-oxadiazole
CAS:	3-Phenyl-5-(2-thienyl)-1,2,4-oxadiazole
CAS Registry No:	330459-31-9
CIPAC No:	996
Synonyms and trade names:	MON 102100
Molecular formula:	C12H8N2OS
Structural formula:	
Molecular mass:	228.27 g/mol

Physical and chemical properties

The information on chemical and physical properties of pure and technical material of tioxazafen are shown in Table 1.

Table 1 The chemical and physical properties of tioxazafen pure and technical material

Property	Results	Reference
Physical form	Cream to light grey Solid	Su 2014 MSL0026308
Melting point	108.6 °C (purity 99.7%)	Su 2014 MSL0026308
Boiling point	Not applicable (purity 99.7%)	van Doormalen 2015 509350
Temperature of decomposition	> 225 °C (purity 99.7%)	van Doormalen 2015 509350
Relative density	0.513–0.674 g/cm³ at 20 °C ± 0.5 °C (purity 99.7%)	Su 2014 MSL0026308
Vapour pressure	$2.52 \times 10^{-5} \pm 5.86 \times 10^{-6}$ Pa at 15 °C $7.76 \times 10^{-5} \pm 1.74 \times 10^{-5}$ Pa at 25 °C $3.66 \times 10^{-4} \pm 5.48 \times 10^{-5}$ Pa at 35 °C (purity 99.4%)	Tunink 2012 MSL0023596
Solubility in water	1.24 mg/L at 20 °C (purity 99.4%, pH 5.93-7.74)	Tunink 2012 MSL0023594

Property	Results	Reference
Solubility in organic solvents	Acetone: 100.48 g/L Dichloromethane 283.88 Ethyl Acetate 105.60 Hexane 6.64 Methanol 11.13 n-Octanol 13.27 Toluene 121.18 (purity 99.7% and tem. at 20±1 °C)	Su 2015 MSL0026668
Octanol/water partition coefficient	log Kow = 4.13 ± 0.15 at 20 °C (pH 6.71-7.87) (purity 99.4%)	Tunink 2012 MSL0023595
pH	6.33 at 22.3°C	Su 2014 MSL0026308
Hydrolysis rate under sterile conditions	Hydrolytically stable at pH 4, 7, and 9 at 50 °C	Elliot 2012 MSL0023830
Direct phototransformation:	DT_{50} = 3.01 h in the pH7 buffer at 25.3±2.4 °C	Hall 2014 MSL0024159
Dissociation constant	No dissociation	Su 2014 MSL0026308
Stability:	No degradation after two weeks at 54°C	van Doormalen 2015 509350
Explodability:	Not explosive	Simmons & Livingston 2014 MSL0025933
Corrosion characteristics:	Corrosive	van Doormalen 2015 509350

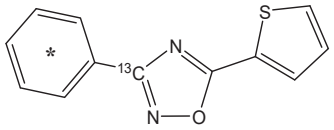
Formulations: Tioxazafen is primarily available as suspension concentrates (SC) for seed treatment containing 541 g ai/L.

METABOLISM AND ENVIRONMENTAL FATE

Radiolabel Position

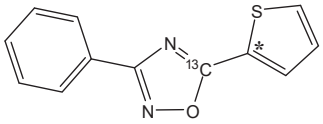
Table 2 Radiolabel Position and Chemical Structure of Test Compound

[oxadiazole-3-^{13}C,phenyl-U-^{14}C]-tioxazafen



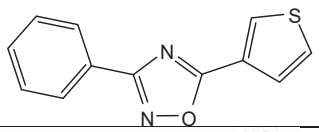
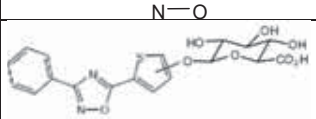
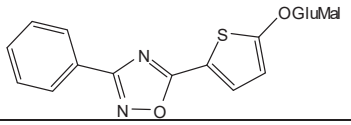
* indicates position of ^{14}C labelled (uniformly ring labelled) PH-T

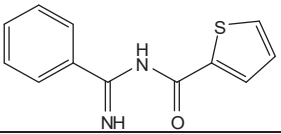
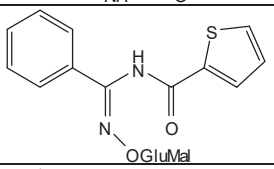
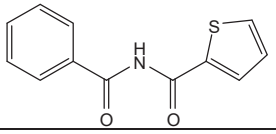
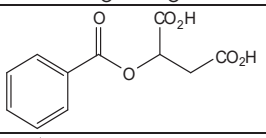
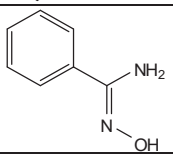
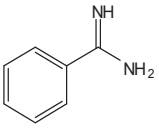
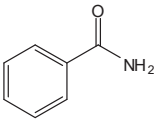
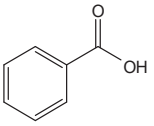
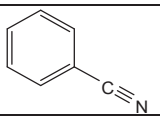
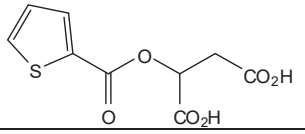
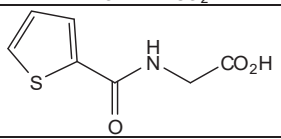
2) [oxadiazole-5-^{13}C,thiophene-2-^{14}C]-tioxazafen

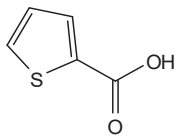
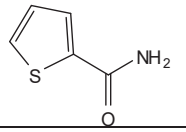


* indicates position of ^{14}C labelled, TH-T

Table 3 Metabolite Codes and Their Related Chemical Structures

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

Trivial Name	Chemical Name	Structure	Where Found
tioxazafen Iminoamide	*N*-(iminophenylmethyl)-2-thiophenecarboxamide		Rotational crops anaerobic aquatic, aerobic aquatic, anaerobic soil
Thenoylbenzamid-oxime 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 carboximidamide		Maize foliage, cotton foliage, goat urine
Benzamidine	benzenecarboximidamide		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, goat urine, goat feces
Benzoic acid	benzoic acid		Maize foliage, cotton foliage, rotational crops; Hen excreta, goat liver, goat skim milk, goat urine, goat feces anaerobic soil
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, goat urine

Trivial Name	Chemical Name	Structure	Where Found
Thiophene acid	2-thiophenecarboxylic acid		Maize foliage, cotton foliage, rotational crops; present as conjugates Hen excreta, goat liver, goat kidney, goat urine, goat feces anaerobic aquatic, aerobic aquatic, anaerobic soil
Thiophene amide	2-thiophenecarboxamide		Maize foliage, rotational crops

Fate and Behaviour in the Environment

Studies of tioxazafen on degradation under aerobic condition, anaerobic condition, hydrolysis and photolysis were received.

Hydrolysis

The hydrolysis of [phenyl-UL-^{14}C] tioxazafen was investigated in sterile aqueous buffer solutions at pH 4 (phthalate buffer), pH 7 (phosphate buffer) and pH 9 (borate buffer) at 50 °C in the dark for 5 days at concentrations of 0.508-0.537µg/mL with acetonitrile (0.45% v/v) as co-solvent (Elliott, 2012 MSL0023830). Tioxazafen is stable to hydrolysis at pH 4, 7 and 9 at 50 °C.

Photo degradation in water

The aqueous photodegradation of [[Phenyl-UL-^{14}C]tioxazafen was investigated in sterile 0.01 M phosphate buffer solution at a temperature of 25 ℃ (Hall, 2014, MSL0024159). In the irradiated conditions, the phototransformation of tioxazafen occurred rapidly, tioxazafen declined from a mean of 99.7% AR at 0-hour to 0.3% AR at 24 hours. A single major photolysis product was identified as 3-thienyl tioxazafen, which reached a mean maximum of 94.0% AR at 10 hours before declining slightly to 91.9% AR at 24 hours. At 24 hours, there were five minor photoproducts, but none of these exceeded 1.8% AR. The calculated phototransformation rate constant, DT_{50}, DT_{75}, and DT_{90} for tioxazafen were 0.230 hour, 3.01 hours, 6.03 hours, and 10.0 hours, respectively. The experimental DT_{50} can be converted to solar equivalent DT_{50} values for various global locations, and these were all <2 hrs. Therefore, direct aqueous photolysis is expected to be a major dissipation route for tioxazafen in aquatic environments, although its planned use as a seed treatment will limit the potential for tioxazafen to reach water bodies. The major photoproduct, 3-thienyl tioxazafen, is expected to be relatively stable to photolysis.

Photodegradation in soil

The photodegradation of tioxazafen was investigated on non-sterile Hoyleton silt loam soil (Sarff and Habeeb 2014 MSL0024160). The soil (pH 7.3, 2.1% organic matter) surface was treated with a solution of radiolabelled tioxazafen at a concentration of 2.76 and 2.85 µg ai/g dry weight soil for the PH- and TH-label samples, corresponding to a field rate of 1.4 kg ai/ha (4.5 times the expected field rate of 0.31 kg ai/ha). The treated samples were placed in a Q-Sun Photolysis Chamber with a xenon arc lamp to simulate sunlight for 15 days at the temperature of 20 ± 2 °C. Sampling was conducted at Day 0, and at 1, 3, 7, 11 and 15 days after treatment, and extracted five times with 65:35 acetonitrile:water. An additional two extractions with 65:35 (v:v) acetonitrile: 0.1 M HCl were conducted on all but the Day 0 samples. At Day 0, unextracted residues were 0.4% of the applied radioactivity (AR), but increased in time, averaging 2.7% AR in the light-exposed samples, and 4.6% AR in the dark control samples at Day 15. Recovery of radioactivity ranged from 94.3% to 104.9% of the applied radioactivity (AR) for the irradiated samples and 97.2% to 110.2% AR for the dark control samples. No significant volatiles were observed in the study (≤0.1% AR). HPLC analysis of the soil extracts showed that there were no significant photoproducts (i.e., ≥5% AR) formed in the light-exposed samples. Only a very small amount (3.0-3.6% AR after 15 days of irradiation) of 3-thienyl 102100 (MON 102130), the photolyte observed as a major phototransformation product in the aqueous photolysis study of tioxazafen, was observed. Except for this minor component, the radioactivity in the extracts was all associated with tioxazafen. The calculation of DT_{50} and DT_{90} values for tioxazafen in irradiated and non-irradiated soil samples was not performed because the results showed virtually no photolysis or degradation of tioxazafen and demonstrate that tioxazafen is photolytically stable on soil surfaces**.**

Field dissipation

The dissipation of tioxazafen and its degradation product benzamidine in soil treated with pre-coated soya bean seeds were studied under field conditions (Jacobson *et al*. 2015 MSL0026630). A companion bare soil plot/experiment with an in-furrow application of the test substance at a rate which simulates the planting of the treated soya bean seed was used to determine dissipation kinetics and mobility potential in soil without a crop. The tests were done in four different North American sites: Tift County, Georgia; York County, Nebraska; Champaign County, Illinois; and Grey Rural Municipality, Manitoba. At each site there were three test plots: one treated seed plot, one treated in-furrow plot, and a control plot. The soils were characterised at each test site. In Georgia, the soil (0-30 cm) was characterised as sand and in Nebraska as clay loam (0-60 cm), in Illinois as clay loam (0-15 cm) and in Manitoba as loamy sand (0-60 cm).

For the treated seed plot the test substance was applied to a commercial variety of soya bean seed as a seed treatment coating at a target rate equivalent to ~0.5 mg ai/seed. In the treated seed plot, the treated soya bean was seeded at a rate of approximately 625,000 seeds/ha (adjusted based on the specific test substance assay results for the treated soya bean varieties) to give an equivalent application rate of 314 g ai/ha for the Illinois and Nebraska test sites, 392 g ai/ha for the Georgia location and 381 g ai/ha for the Manitoba location.

The treated in-furrow plot received a single in-furrow application of the test substance formulated as MON 102119 (573 g ai/L SC for seed treatment) at a rate of 0.31 kg ai/ha which simulated the planting of treated seed at the rate and planting population in the treated seed plot. The application (treated seed and in-furrow) at each test site was timed to occur at the approximate typical timing of soya bean planting for that location/region.

Test site description is detailed in Table 4.

Table 4 Soil Properties (0-15 cm) at the different sites

Soil property	Tift Co. Georgia	York Co. Nebraska	Champaign County, Illinois	Grey Rural Municipality, Manitoba
Sand % (0.05-2 mm)[a]	88	26	21	85
Silt %(0.002-0.05mm)[a]	9	45	49	7
Clay % (<0.002 mm)[a]	3	29	30	8
pH (water, 1:1)	6.7	7.6	6.6	8.5
% Organic matter[b]	0.72	3.1	4.1	2.9
C.E.C [meq/100g][c]	4.2	20.4	18.8	14.1
Bulk density disturbed (g cm^{-3})	1.49	1.15	1.15	1.17
Bulk density undisturbed (g cm^{-3})[d]	1.76	1.37	1.22	1.35
Moisture Holding Capacity at 1/3 bar (% of dry weight)	5.2	29.7	32.3	12.8
Moisture Holding Capacity at 15 bar (% of dry weight)	2.1	15.6	16.0	6.9
Soil Classification[e]	Sand	Clay loam	Clay loam	Loamy sand

a) Particle size

b) Walkley-Black method

c) Cation Exchange Capacity (C.E.C)

d) Average of three determinations

e) Soil classification according to USDA system

At each location, soil cores were targeted for collection in the treated plots prior to the application (PA), and 0 DAA (immediately after the application), 1, 3, 7, 14, 21, 28, 60, 90, 120, 150, 180, 270, 360, 450 and 540 days after application (DAA). Soil cores were sectioned/separated into segments with core segments composited by depth for each replicate subplot.

Soil samples were analysed through the 360 DAA sampling event from the treated plots in Georgia, Nebraska, Illinois and the treated in-furrow plot in Manitoba and through 540 DAA in the treated seed plot in Manitoba for residues of parent, tioxazafen (3-phenyl-5-thiophen-2-yl-1,2,4-oxadiazole), and the soil degradation product benzamidine. Residues of tioxazafen and its degradation product benzamidine were analysed separately in study soil. Benzamidine residues are expressed as benzamidine per se, not parent equivalents.

Residues of tioxazafen remained primarily in the 0–15 cm soil layer in both treated plots at all four locations. In the treated seed plot there were no tioxazafen residues found at or above the LOQ (0.0050 mg/kg) in any samples below the 0–15 cm

depth at the Georgia, Nebraska or Illinois test sites. Tioxazafen was only found above the LOQ in two replicate samples (0.0069 and 0.0056 mg/kg) in the 15-30 cm depth at 3 DAA at the Manitoba site. There were no tioxazafen residues found at or above the LOQ in any samples below the 15-30 cm inch depth.

In the treated in-furrow plot there were no tioxazafen residues found at or above the LOQ in any samples below the 0-15 cm depth at the Georgia test site. At the Nebraska, Illinois and Manitoba test sites residues of tioxazafen were only found in the 15-30 cm layer sporadically at low levels ranging from 0.0060 to 0.0483 mg/kg. There were no tioxazafen residues found at or above the LOQ in any samples below the 15-30 cm depth.

There were no residues of benzamidine found at or above the LOQ (0.0013 mg/kg) in any samples below the 0–7.5 cm depth in both treated plots at all four test sites, except for one replicate sample at the Manitoba site on 492 DAA at the 15-30 cm depth at a level of 0.0015 mg/kg. Benzamidine residues were found sporadically in the 0 to 7.5 cm depth at low levels at all four sites ranging from 0.0014 to 0.0243 mg/kg.

Dissipation of tioxazafen was assessed using first-order (SFO) and biphasic (FOMC/IORE, DFOP) kinetic models. The total mass of tioxazafen in the 0-30 cm sampled soil profile (deepest depth with residues above the LOQ) was used in the kinetic calculations. Basing kinetic calculations on total mass in the soil profile better reflects dissipation processes, not just simple topsoil dissipation. The pattern of tioxazafen decline at all four sites suggested a slowing of the dissipation rate during the fall/winter months, consistent with the effect of cooling temperatures on a biological process such as aerobic microbial degradation. Dissipation of tioxazafen occurred at a moderate rate in the treated seed plots and treated in-furrow plots at all four sites. The DT50 values of the best-fit models at the Georgia, Nebraska, Illinois and Manitoba for both treated plots are presented below.

In the treated seed plot, the DT_{50} values ranged from 15 days to 289 days with a median of 70 days, an average of 111 days, and a standard deviation of 107 days. In the treated in-furrow plot, the DT_{50} values ranged from 40 to 101 days with a median of 89 days, an average of 80 days, and a standard deviation of 23 days. Results are summarised below in Table 5.

Table 5 Summary of Kinetics from Treated Seed and In-Furrow Treatments

Site	Plot	Kinetic model	DT_{50} (days)	DT_{75} (days)	DT_{90} (days)
Georgia	Treated seed	SFO	94.3	189	313
	In furrow	FOMC/IORE	40.1	116	332
Nebraska	Treated seed	DFOP	14.7	136	315
	In furrow	SFO	101	202	336
Illinois	Treated seed	SFO	44.7	89.4	149
	In furrow	SFO	90.5	181	301
Manitoba	Treated seed	SFO	289	578	960
	In furrow	SFO	87.3	175	290

The residue concentrations (in mg/kg) in the 0–30 cm soil profile were converted to total g /ha for non-linear regression analysis and the calculation of kinetic endpoints. The SFO, DFOP or IORE/FOMC kinetics models provided the best fits to the total system (0–30 cm) replicate residue data for all treated plots at all locations.

The pattern of tioxazafen decline at all four sites suggested a slowing of the dissipation rate during the fall and winter months, consistent with the effect of cooling temperatures on a biological process such as aerobic microbial degradation. Dissipation of tioxazafen occurred moderately in the treated seed plot at all four sites. The DT_{50} values of the selected models at Georgia, Nebraska, Illinois and Manitoba treated seed plots were 94.3, 14.7, 44.7, and 289 days, respectively. Dissipation of tioxazafen occurred at a moderate rate in the treated in-furrow plot at all four sites. The DT_{50} values of the selected models at Georgia, Nebraska, Illinois, and Manitoba in-furrow treated plots were 40.1, 101, 90.5, and 87.3 days, respectively.

Kinetic models were not fit to the degradation product benzamidine data due to the low and/or variable levels observed. The sampling event DAA that residues first appeared above the LOQ, the maximum mg/kg observed, sampling event DAA of maximum mg/kg observed, and the sampling event DAA the concentration reached less than LOQ for each treated plot at the four test sites can be found summarised in Table 6.

Table 6 Summary of Observed Benzamidine Residues for All Treated Plots in Each of the Test Sites at Georgia, Nebraska, Illinois, and Manitoba

Site	Plot	First Appeared >LOQ (DAA)	Max (mg/kg)[a]	Max (DAA)[b]	Reached <LOQ (DAA)
Georgia	Treated Seed	14	0.0117	92	272
	Treated In-Furrow	92	0.0014	92	121
Nebraska	Treated Seed	0	0.0063	62, 92	310

Site	Plot	First Appeared >LOQ (DAA)	Max (mg/kg)[a]	Max (DAA)[b]	Reached <LOQ (DAA)
	Treated In-Furrow	28	0.0053	120	154
Illinois	Treated Seed	14	0.0071	90	NR[c]
	Treated In-Furrow	120	0.0016	120	151
Manitoba	Treated Seed	28	0.0070	115	NR
	Treated In-Furrow	28	0.0015	28	136

a) Max = maximum average level of n=3 replicates (mg/kg, dry weight).
b) DAA = Days after application.
c) NR = Not reached.

The DT_{90} values for tioxazafen dissipation in Georgia, Nebraska, Illinois, and Manitoba for both treated seed and in-furrow treatment plots were less than a year except in treated seed plots in Manitoba in which the DT_{90} was 960 days. Therefore, the potential for significant amounts of tioxazafen to carry over into the following season is relatively low. Additionally, carry-over of the tioxazafen transformation product, benzamidine, will be insignificant.

Degradation in aerobic soils.

The degradation of PH- or TH-labelled tioxazafen was evaluated in three soils (Hoyleton silt loam, Webster sandy clay loam and Barnes-Svea clay loam) in incubations for four months at 20 °C in the dark (Shepler 2013 MSL0024420). The soils were treated with radiolabelled tioxazafen at a target rate of 0.62 mg/kg, based on a field application rate of 0.31 kg ai/ha. The actual treatment rate was 0.621 mg/kg dry soil in the PH-labelled treatments and 0.638 mg/kg dry soil in the TH-labelled treatments. The soils were maintained at 20 °C at a moisture level intermediate between pF 2.0 to 2.5. Duplicate samples were taken at Time 0 and at an additional seven or eight time points, dependent upon the soil, over the four-month incubation period. The soils were extracted with 65:35 (v/v) acetonitrile/water three times, and then an additional two extractions with the same solvent were conducted on all but the Time 0 samples because significant levels of the applied radiocarbon remained unextracted after three extractions. Non-extracted radioactivity increased through the incubation period reaching 46.6-47.3%, 33.5-34.2% and 26.4-27.6% of the applied radioactivity (AR) at the end of the study for Hoyleton silt loam, Webster sandy clay loam and Barnes-Svea clay loam, respectively. The average overall recoveries over the course of the study were 98.0 ± 2.3%, 99.0 ± 3.0% and 100.0 ± 3.1% for the Hoyleton, Webster and Barnes-Svea soils, respectively, for the PH-label set, and 96.7 ± 3.5%, 98.7 ± 1.8% and 100.1 ± 2.1% for the corresponding soils in the TH-label set.

With the exception of the TH-label set in the Hoyleton silt loam, the dissipation of tioxazafen was characterised by a rapid initial decline of approx. 10-20% over the first 3-5 days of incubation (presumably due to soil binding) followed by a slower dissipation phase. This biphasic behavior was most evident in the Webster and Barnes-Svea soils, and was not adequately described using the SFO model. The FOMC model provided a good fit for the data for the Hoyleton silt loam, but was not selected because this model is considered inappropriate for data sets that do not reach or approach 90% dissipation at the end of the study (FOCUS, 2006). Using the SFO model (with fixed Time 0 values for the PH-label data), it was found that dissipation of tioxazafen proceeded at a moderate rate in the Hoyleton silt loam with DT_{50} values of 50.8 days (PH label) and 57.1 days (TH label) and corresponding DT_{90} values of 169 and 190 days (extrapolated beyond end of study). Dissipation in the Webster sandy clay loam and Barnes-Svea clay loam was considerably slower with DT_{50} values of 141/144 days and 221/277 days, respectively. Calculated DT_{90} values (extrapolated beyond the end of the study) ranged from 524 days to >1000 days for these two soils. The hockey-stick model gave a much better fit than the SFO model for the Webster and Barnes-Svea soils in which the biphasic dissipation was more pronounced.

The aerobic rate of dissipation of tioxazafen in soil did not correlate well with soil biomass (r^2=0.17) for the four soils in which the aerobic metabolism of tioxazafen was evaluated and the three soils described herein. The rate of dissipation did appear to be highly correlated (r^2=0.99) inversely with clay content of the soils (longer DT_{50} values in soils with higher clay content).

The principal routes of dissipation of tioxazafen were formation of bound residues and mineralisation to $^{14}CO_2$. At the end of the incubation period, released $^{14}CO_2$ amounted to 18.2-19.2% AR, 5.6% AR and 3.2-3.5% AR for Hoyleton silt loam, Webster sandy clay loam and Barnes-Svea clay loam, respectively. There were no metabolites observed in the extracts of the three soils that corresponded to synthetic reference standards of potential metabolites of tioxazafen. Other very minor unidentified components in the soil extracts were observed only sporadically and represented, in total, ≤2.6% AR in any individual sample at any time point.

PH-label Hoyleton silt loam

PH-label Webster sandy clay loam

PH-label Barnes-Svea clay loam

Figure 1 Dissipation of ^{14}C-tioxazafen in soils (PH-label)

TH-label Hoyleton silt loam

TH-label Webster sandy clay loam

TH-label Barnes-Svea clay loam

Figure 2 Dissipation of ^{14}C-tioxazafen in soils (TH-label)

Error! Reference source not found. Table 7 provides the results of that fractionation showing that the majority of the unextracted soil residue was found in the humin for all three soils. The non-extractable residues were characterised by fractionation into humic acids, fulvic acids and humin. The distribution into fulvic acids, humic acids and humin is similar for both labels in each soil, but the distribution is quite different in the Hoyleton silt loam, and the Manning sandy loam route soil, compared to the Webster sandy clay loam and Barnes-Svea clay loam in which the dissipation of tioxazafen was much slower.

Table 7 Fractionation of the Non-Extractable Soil Radioactivity into Fulvic Acids, Humic Acids and Humin

	Distribution of the Radioactivity in the Unextracted Soil Residues (Percent)			
PH Label	Sample	Fulvic Acids	Humic Acids	Humin
Hoyleton silt loam	A	11.6	5.9	16.9
	B	12.3	6.0	16.5
	Mean	12.0	6.0	16.7
Webster sandy clay loam	A	4.8	1.8	12.8
	B	4.9	1.8	9.6
	Mean	4.9	1.8	11.2
Barnes-Svea clay loam	A	1.0	0.5	12.6
	B	0.9	0.6	12.6
	Mean	1.0	0.6	12.6
TH Label	Sample	Fulvic Acids	Humic Acids	Humin
Hoyleton silt loam	A	10.1	7.6	19.6
	B	8.7	6.2	21.5
	Mean	9.4	6.9	20.6

	Distribution of the Radioactivity in the Unextracted Soil Residues (Percent)			
PH Label	Sample	Fulvic Acids	Humic Acids	Humin
Webster sandy clay loam	A	3.0	1.9	16.6
	B	2.7	1.7	13.1
	Mean	2.9	1.8	14.9
Barnes-Svea clay loam	A	0.9	0.7	10.2
	B	0.9	0.8	11.8
	Mean	0.9	0.8	11.0

Over a four-month incubation period, levels of tioxazafen decreased to 28.1% AR for the phenyl- labelled tioxazafen and 23.5% AR for the thiophene-labelled tioxazafen in the extracts of the Hoyleton silt loam soil. In the Webster and Barnes-Svea soils, dissipation proceeded more slowly with an average of 53.2% and 57.4% tioxazafen remaining at the end of the incubation in the extracts of the PH and TH samples of the Webster sandy clay loam soil. In the extracts of the PH and TH samples of the Barnes-Svea clay loam, tioxazafen represented an average of 67.7 and 67.9% AR at the end of the incubation.

The data showing the DT_{50} and DT_{90} values obtained using the kinetic model with the best fit for each soil and each label, and the results from the initial SFO calculations are shown in **Error! Reference source not found.**8. The kinetic analysis producing the best fit to the data is shown in bold font; only in the case of the TH-label in Hoyleton silt loam was the single first-order analysis the best fit to the data.

Table 8 DT_{50} and DT_{90} of tioxazafen in Laboratory Aerobic Incubation in Four Soils

Soil	Test Substance	DT_{50} (days)	DT_{90} (days)	Chi^2 Error (%)	r [b]	Kinetic Model [a]
Hoyleton silt loam	PH-^{14}C	55.3	184	5.1294	0.9732	SFO
		50.8	169	6.2108	0.9762	SFO (fixed T0)
	TH-^{14}C	57.1	190	1.7594	0.9890	SFO
Webster sandy clay loam	PH-^{14}C	152	504	3.5548	0.9378	SFO
		141	524	2.0476	0.9788	HS
	TH-^{14}C	151	501	3.0868	0.9507	SFO
		144	534	1.3700	0.9911	HS
Barnes-Svea clay loam	PH-^{14}C	235	780	4.8197	0.7656	SFO
		221	888	1.6030	0.9380	HS
	TH-^{14}C	252	836	3.8673	0.8064	SFO
		277	>1000	2.0523	0.9341	HS
Manning sandy loam soil[2]	PH-^{14}C	34.3	114	10.5278	0.9283	SFO
		23.5	280	6.8326	0.9653	FOMC
	TH-^{14}C	27.2	90.4	7.9311	0.9702	SFO
		20.2	159	4.3906	0.9898	FOMC

[a] SFO = single first-order; HS = hockey-stick; SFO (fixed T0) = single first-order with Time 0 values fixed to the average of the observed Time 0 values; FOMC = first-order multi-compartment.

[b] Data from aerobic route of degradation study summarised in Shepler 2013;

Plant metabolism

The meeting received information on metabolism studies of tioxazafen, labelled either in phenyl or thiophene ring, after seed treatment (representative use pattern) in soya bean, maize and cotton.

Soya bean (Categories pulse and oilseeds)

Soya bean seeds (*Glycine max* L., Asgrow® AG4606, Roundup Ready STS, tolerant to glyphosate and acetolactate synthase inhibitor herbicides) were treated with [phenyl-U-^{14}C]-tioxazafen (PH-T) or [thiophene-2-^{14}C]-tioxazafen (TH-T) (Kurtzweil *et al*., 2014 MSL0023358) formulated as an aqueous suspension concentrate, and applied directly on the seeds using procedures designed to closely mimic commercial seed treatment. The treatment rates were 1.3 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 and control soya bean seeds were planted outdoors in 0.9 × 0.9 m wooden boxes lined with plastic and filled to a depth of approximately 0.46 m with loamy sand soil (pH 7.1, 0.64% organic matter). Samples of thinnings (28 days after planting,

BBCH 12), forage (48 days after planting, BBCH 17), hay (88 days after planting) and seed (147 days after planting) were collected and stored frozen. Samples were maintained frozen through processing (homogenising and cryomilling).

Samples of thinnings were extracted three times with 80:20 (v/v) acetone: water, once with 40:60 acetone: water, and twice with 20:80 acetone: water. Forage and hay samples were extracted four times with 40:60 acetone: water. Seed samples were extracted three times with hexane to remove oils, then once with acetone followed by four extractions with 40:60 (v/v) acetone: water. The post-extraction solids (PES) remaining after 40:60 (v/v) acetone: water extractions of the PH-T and TH-T soya bean forage and hay were subjected to additional acid and base treatments, enzymatic digestions, and chemical digestions to release unextracted residues.

Radioactivity was measured by combustion and LSC analysis of the $^{14}CO_2$ trapping solutions. Characterisation and idenfication of metabolites were conducted with HPLC-RAD (radioactive flow detector) or UV, HPLC-LSC or UV, LC-MS/MS or GC-MS. Comparison of the chromatographic and mass spectral properties of the metabolite derivatives or hydrolysates to those of synthetic reference standards was used to confirm the identities of the key metabolites.

Residue levels (expressed as parent tioxazafen equivalents) were highest in thinnings, then decreased substantially in forage and hay, and were lowest in seed. Residue levels for a given matrix were generally comparable for the two labels. Thinnings (immature foliage) harvested 28 days after planting gave TRRs of 9.05 mg eq/kg and 10.9 mg eq/kg for the PH-T and TH-T labels, respectively. Forage harvested 48 days after planting gave TRRs of 0.426 mg eq/kg (PH-T) and 0.510 mg eq/kg (TH-T), while hay harvested 88 days after planting gave slightly higher TRRs of 0.779 mg eq/kg (PH-T) and 1.06 mg eq/kg (TH-T). Residue levels in the seed from both labels collected 147 days after planting were considerably lower than those in forage or hay indicating limited translocation of tioxazafen residues to the seed. Residue levels in PH-T seed were 0.0696 mg eq/kg compared with residues of 0.165 mg eq/kg in TH-T seed. The results are summarised in Table 9.

Table 9 Total radioactive residues in different matrices of soya bean treated with PH-T or TH-T

Matrix	Days After Planting	TRR of PH-T mg eq/kg	TRR of TH-T mg eq/kg
Thinnings	28	9.05	10.9
Forage	48	0.426	0.510
Hay	88	0.779	1.06
Seed	147	0.0696	0.165

Acetone:water extracted 69.1-74.1% TRR from thinnings, 55.6-62.2% TRR from forage and 52.1-56.0% TRR from hay. About 70% TRR in seed was extracted with hexane, followed by acetone and acetone: water. The results of the extraction and analysis are provided in Table 10 for the PH-T and in Table 11 for the TH-T label.

Because the TRRs in the thinnings were much higher than that in the forage and hay, the metabolites of tioxazafen in thinnings were identified. Metabolism of tioxazafen in thinnings was very complex, resulting in 34 and 59 integrated peaks or regions (less polar than tioxazafen) for the PH-T label and TH-T label quantitation profile. Only one peak exceeded 10% of the TRR for either label, two other peaks exceeded 5% of the TRR for the PH-T label and one other peak exceeded 5% of the TRR for the TH-T label. The major metabolite in soya bean PH-T thinnings was benzamidine (10.6% of TRR). Benzamidine was also the most abundant metabolite in soya bean PH-T forage, hay, and seeds at 8.5%, 8.1%, and 10.9% TRR, respectively. The next most abundant metabolite in PH-T thinnings was benzoylmalic acid constituting 8.5% of TRR. Tioxazafen in PH-T thinnings was at a level of 5.6% TRR. Tioxazafen was at a level of 4.7% of TRR in TH-T thinnings.

Three other metabolites were either characterised or identified in PH-T thinnings. One of the metabolites, present at 5.0% TRR, with a nominal molecular mass of 365 was converted to benzoic acid under strong base hydrolysis; however, a reasonable structure could not be assigned. The other two metabolites identified in PH-T thinnings were also identified in TH-T thinnings after acid and base hydrolysis. Thenoylbenzamidoxime malonylglucoside, was present at 3.8% and 4.1% TRR for the PH-T and TH-T labels, respectively. The other identified metabolite common to both labels was hydroxy (thiophene) tioxazafen malonylglucoside, which was present at levels of 4.3% and 4.9% TRR for the PH-T and TH-T labels, respectively. In addition to the two malonylglucoside metabolites identified in TH-T thinnings, two other metabolites were either characterised or identified. One of these was identified as thenoylmalic acid, which was present at 3.6% TRR. The other metabolite (5.6% TRR) was not identified, but was characterised as having a modified thiophene ring based on strong acid and strong base hydrolysis reactions.

In all, identified or characterised metabolites constituted 37.8% TRR in PH-T thinnings and 22.9% TRR in TH-T thinnings. The low percentage of identified or characterised residues is not only due to the complex metabolism of tioxazafen in thinnings, but also the relatively large proportion of unextracted residues for both labels (25.9% and 30.9% TRR for PH-T and TH-T labels, respectively).

Table 10 Summary of the Extraction, Characterisation and Identification of the Residues of Tioxazafen in Soya bean Matrices from the PH Label

Compound	Thinnings TRR 9.05 mg eq/kg		Forage TRR 0.426 mg eq/kg		Hay TRR 0.779 mg eq/kg		Seed TRR 0.0696 mg eq/kg	
	%	mg eq/kg	%	mg eq/kg	%	mg eq/kg	%	mg eq/kg
Acetone/water extracts*	74.1	6.71	62.2	0.265	56.0	0.436	62.1	0.0485
Hexanes extracts	NA	NA	NA	NA	NA	NA	7.5	0.0052
Benzamidine	10.6	0.955	8.5	0.0362	8.1	0.0631	10.9	0.0076
Unknown, MW 365	5.0	0.449	NQ	NA	NQ	NA	NQ	NA
Benzoylmalic acid	8.5	0.772	NQ	NA	NQ	NA	NQ	NA
Thenoylbenzamidoxime malonylglucoside	3.8	0.346	2.7	0.0113	2.41	0.0188	NQ	NA
Hydroxy (thiophene) Tioxazafen Malonylglucoside	4.3	0.386	1.7	0.0073	0.9	0.0067	0.6	0.0004
Tioxazafen	5.6	0.510	12.5	0.0532	4.3	0.0337	≤0.9**	≤0.0006**
Total identified	37.8	3.42	25.3	0.108	15.7	0.122	12.4	0.0086
Total unextracted	25.86	2.34	37.79	0.161	44	0.343	30.38	0.0212
0.1 N HCl			1.60	0.0068	1.56	0.0122		
0.1 N NaOH			4.82	0.0206	2.96	0.0230		
Phosphate			1.55	0.0066	1.41	0.0109		
Starch			0.84	0.0036	0.93	0.0073		
Protein			1.98	0.0084	2.40	0.0187		
Pectin			1.30	0.0055	2.19	0.0171		
Lignin			15.70	0.0669	17.81	0.1387		
Cellulose			2.98	0.0127	1.64	0.0128		
Hemicellulose			6.91	0.0295	12.23	0.0952		
Total released			99.87		99.13			

NQ – Not Quantified (not present or too small to quantify), NA – Not Applicable

* The extractability is based on normalized values (sum of the extractable and non-extractable radioactivity).

** Tioxazafen was not actually identified in the hexane extract. Values based on percentage of radioactivity extracted into acetonitrile from the hexanes extracts.

Table 11 Summary of the Extraction, Characterisation and Identification of the Residues of Tioxazafen in Soya bean Matrices from the TH Label

Compound	Thinnings TRR 10.9 mg eq/kg		Forage TRR 0.510 mg eq/kg		Hay TRR 1.06 mg eq/kg		Seed TRR 0.165 mg eq/kg	
	%	mg eq/kg	%	mg eq/kg	%	mg eq/kg	%	mg eq/kg
Acetone/water extract*	69.1	7.56	55.6	0.284	52.1	0.553	62.69	0.115
Hexanes extract	NA	NA	NA	NA	NA	NA	7.0	0.0116
Unknown	5.6	0.614	NQ	NA	NQ	NA	NQ	NA
Thenoylmalic acid	3.6	0.395	NQ	NA	NQ	NA	NQ	NA
Thenoylbenzamidoxime malonylglucoside	4.1	0.445	2.9	0.0147	2.7	0.0283	NQ	NA
Hydroxy (thiophene) Tioxazafen Malonylglucoside	4.9	0.537	4.4	0.0224	1.1	0.0114	NQ	NA
Tioxazafen	4.7	0.514	5.0	0.0256	5.1	0.0544	≤0.5**	≤0.0008**
Total identified	22.9	2.51	12.3	0.0627	8.9	0.0942	≤0.5**	≤0.0008**
Total unextracted	30.9	3.38	44.4	0.226	47.9	0.509	30.26	0.0498
0.1 N HCl			2.34	0.0119	1.63	0.0173		
0.1 N NaOH			5.02	0.0256	3.58	0.0380		
Phosphate			1.42	0.0072	1.48	0.0157		
Starch			0.82	0.0042	1.08	0.0115		
Protein			2.85	0.0145	2.99	0.0318		
Pectin			1.03	0.0052	1.79	0.0190		
Lignin			16.16	0.0824	15.97	0.1697		
Cellulose			3.54	0.0180	1.91	0.0203		
Hemicellulose			8.66	0.0442	14.54	0.1545		

Compound	Thinnings TRR 10.9 mg eq/kg		Forage TRR 0.510 mg eq/kg		Hay TRR 1.06 mg eq/kg		Seed TRR 0.165 mg eq/kg	
	%	mg eq/kg	%	mg eq/kg	%	mg eq/kg	%	mg eq/kg
Total released			97.43		97.07			

NQ – Not Quantified (not present or too small to quantify), NA – Not Applicable

* The extractability is based on normalized values (sum of the extractable and non-extractable radioactivity).

** Tioxazafen was not actually identified in the hexanes extract. Values bases on percentage of radioactivity extracted into acetonitrile from the hexanes extracts.

The post-extracted solids (PES) obtained after solvent extraction of forage and hay were used to investigate the release of unextracted residues through treatment with acid and base, and a series of chemical and enzymatic digestions. Aliquots of forage and hay PES samples were initially extracted with dilute acid and dilute base. Subsequent chemical and enzymatic digestions were performed to release starch, protein, pectin, lignin, cellulose, and hemicellulose. Dilute acid or base extraction released very little (≤5% TRR) of the unextracted residues from either matrix. The chemical and enzymatic digestions to release starch, protein, pectin, and cellulose also released very little of the unextracted residues (≤4% TRR). More substantial residues were released along with the lignin (15.7-17.8% TRR) and hemicellulose (6.9-14.5% TRR) fractions. Partitioning of the lignin fraction with ethyl acetate resulted in transfer of a majority of the radioactive residues into the organic phase for all samples, while partitioning of the hemicellulose fraction with ethyl acetate resulted in retention of the majority of residues in the aqueous phase. The most significant amount of residue was released into the aqueous phase of the TH-T hay, indicating that the residues are more polar.

Table 12 Summary of Organic Solvent Partitioning of Enzymatic and Chemical Digests of PES in Soya bean Forage

Fraction	PH-T Forage		TH-T Forage	
	Percent Partitioned	mg eq/kg*	Percent Partitioned	mg eq/kg*
Lignin		0.0669		0.0824
Aqueous	19.98	0.0134	27.28	0.0225
Organic	75.94	0.0508	37.58	0.0310
Hemicellulose		0.0295		0.0442
Aqueous	77.69	0.0229	31.27	0.0138
Organic	17.28	0.0051	39.91	0.0176

Table 13 Summary of Organic Solvent Partitioning of Enzymatic and Chemical Digests of Unextracted Residues in Soya bean Hay

Fraction	PH-T Hay		TH-T Hay	
	Percent Partitioned	mg eq/kg*	Percent Partitioned	mg eq /kg*
Lignin		0.1387		0.1697
Aqueous	24.53	0.0340	33.37	0.0566
Organic	64.61	0.0896	38.78	0.0658
Hemicellulose		0.0952		0.1545
Aqueous	64.74	0.0616	78.63	0.1215
Organic	36.42	0.0347	21.22	0.0328

*tioxazafen equivalent

Table 14 Summary of Hydrolysis of the Aqueous Partitioning Phase of Hemicellulose Digest of Unextracted Residues for TH-T Hay followed by Organic Solvent Partitioning

Fraction	Percent Partitioned	Percent of TRR	mg eq/kg*
Hemicellulose**		11.43	0.1215
Aqueous	16.90	1.93	0.0205
Organic	10.24	1.17	0.0124

*Tioxazafen equivalents

**The aqueous phase from the hemicellulose digest was neutralized, and then adjusted to 2 M HCl before heating at 100 °C for 2 hours.

Table 15 Summary of HPLC of Organic Phase of Lignin Digest of Unextracted Residues for PH-T Forage and Hay Partitioning Using HPLC Method A

Fraction	PH-T Forage			PH-T Hay		
	Area Percent HPLC Peak	Percent of TRR	mg eq /kg*	Area Percent HPLC Peak	Percent of TRR	mg eq /kg*
Lignin Partitioning						
Organic Phase		11.92	0.0508		11.51	0.0896
HPLC Peak Number						
1	3.61	0.43	0.0018	4.86	0.56	0.0044
2	4.76	0.57	0.0024	8.98	1.03	0.0080
3	6.20	0.74	0.0032	6.89	0.79	0.0080
4	38.11	4.54	0.0194	41.55	4.78	0.0372
5	11.24	1.34	0.0057	7.40	0.85	0.0066
6	1.04	0.12	0.0005	NA	NA	NA

*Tioxazafen equivalents

To summarise, in all, five discrete metabolites of tioxazafen and unmetabolized parent tioxazafen were identified. Tioxazafen was present at 5.6% TRR (0.510 mg eq/kg) and 4.7% TRR (0.514 mg eq/kg) in PH-T and TH-T thinnings, respectively. Parent tioxazafen in forage and hay was at levels ranging from 4.3-12.5% TRR (0.0256-0.0544 mg eq/kg). Parent tioxazafen in seed was very minor (less than 0.9% TRR, <0.0008 mg eq/kg) component for both labels. The major metabolite identified in the PH-T soya bean matrices was benzamidine, which constituted 10.6% (0.955 mg eq/kg), 8.5% (0.0362 mg eq/kg), 8.1% (0.0631 mg eq/kg), and 10.9% (0.0076 mg eq/kg) TRR in thinnings, forage, hay and seed, respectively. Three other significant metabolites identified in PH-T thinnings were: benzoylmalic acid (8.5% TRR, 0.772 mg eq/kg), thenoylbenzamidoxime malonylglucoside (3.8% TRR, 0.346 mg eq/kg), and hydroxy (thiophene) tioxazafen malonylglucoside (4.3% TRR, 0.386 mg eq/kg).

A proposed pathway for metabolism of tioxazafen in soya bean plants based on identified/characterised metabolites is presented below in Figure 3.

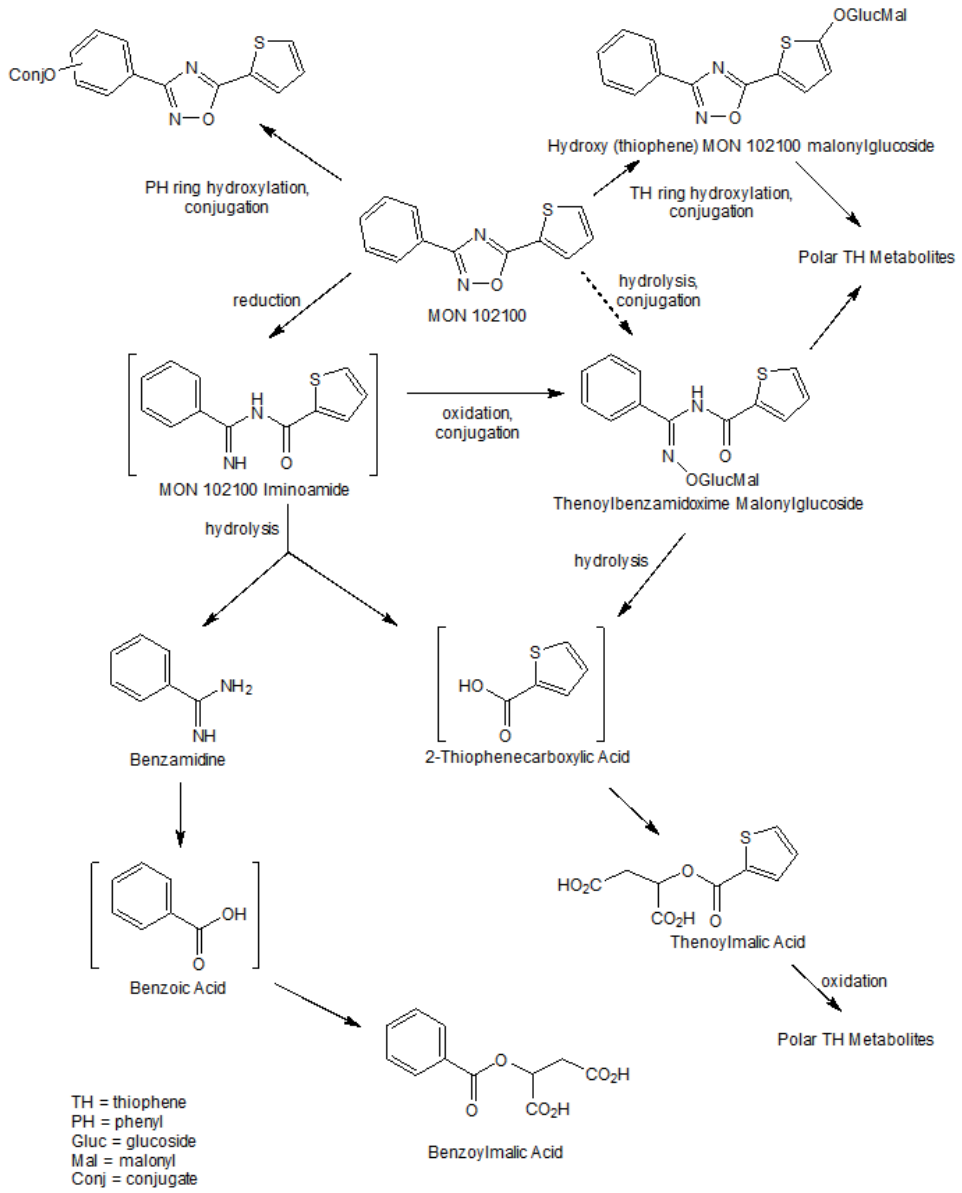


Figure 3 Proposed Metabolic Pathway for Tioxazafen in Soya bean

Maize (Categories cereals)

A metabolism study was conducted using two different radiolabelled [^{14}C] tioxazafen applied to separate batches of maize seed to determine the nature of residues in/on maize and to identify the pathway of tioxazafen metabolism in maize following seed treatment (Quistad and Kovatchev 2014 MSL0023356). Maize seeds (*Zea mays* L., Dekalb® DKC69-71, a RoundupReady® Corn 2/YieldGard® Corn Borer variety, tolerant to glyphosate and acetolactate synthase inhibitor herbicides) were treated with [oxadiazole-3-^{13}C, phenyl-UL-^{14}C] tioxazafen or [oxadiazole-5-^{13}C, thiophene-2-^{14}C] tioxazafen (TH-T). Labelled tioxazafen was separately formulated and applied directly on the seeds using formulation components and procedures simulating commercial use. The application rates were 1.09 mg ai/seed (0.26 kg ai/ha, 109% of target) for the PH-T treatment and 1.28 mg ai/seed (0.30 kg ai/ha, 128% of target) for the TH-T treatment.

Treated maize seeds were planted outdoors in 1.2 × 1.2 m wooden boxes lined with plastic and filled to a depth of approximately 0.46 m with loamy sand soil (pH 7.1, 0.64% organic matter). At the appropriate growth stage, immature foliage samples were collected as thinnings 24 days after planting. Forage samples were collected 101 days after planting. Stover

and grain was collected 130 days after planting. Specimens were frozen promptly after collection, and were maintained frozen through processing (homogenization) until they were withdrawn for analysis. The plant matrices were subjected to an exhaustive extraction procedure, involving three extractions with 80:20 acetone/water followed by an extraction with 40:60 acetone/water for thinnings, four extractions with acetone/water 40:60 for forage, stover and grain. Acid and base treatments of acetone/water extracts were used to cleave conjugates to release the exocons for analysis. If PES contained >10% TRR, digestions with 0.1 M KOH or 24% KOH were conducted. Radioactivity was measured by combustion and LSC analysis of the $^{14}CO_2$ trapping solutions. Characterisation and idenfication of metabolites was primarily conducted using HPLC-LSC. TLC was used as an aid in metabolite identification by comparing retention times of metabolites with reference standards.

Residues were low in all matrices, except for the thinnings. The TRRs were 1.719 mg eq/kg (PH-T) and 1.967 mg eq/kg (TH-T) in thinnings, 0.0148 mg eq/kg (PH-T) and 0.0084 mg eq/kg (TH-T)) in forage, 0.0644 mg eq/kg (PH-T) and 0.0415 mg eq/kg (TH-T) in stover, 0.0012 mg eq/kg (PH-T) and 0.0020 mg eq/kg (TH-T) in grain. Residue levels were comparable for the two radiolabels.

Table 16 Total radioactive residues in different matrices of maize treated with PH-T or TH-T

Matrix	Days After Planting	TRR of PH-T (mg eq/kg)	TRR of TH-T (mg eq/kg)
Thinnings	24	1.719	1.967
Forage	101	0.0148	0.0084
Stover	130	0.0644	0.0415
Grain	130	0.0012	0.0020

The solvent systems used (acetone:water) extracted 83.3-84.4% of TRR in thinnings, 67.9-71.0% of TRR in forage, 66.8-67.8% of TRR in stover and 11.6-41.9% of TRR in grain. The TRR in maize grain was very low for both labels with only 0.0005 mg eq/kg and 0.0002 mg eq/kg for the PH and TH maize grain, respectively. HPLC analysis was not feasible for grain due to the low levels of radioactivity in these extracts.

Table 57 Summary of the Extraction, Characterisation and Identification of the Residues of Tioxazafen in Maize Matrices from the PH Label

Compound	TRR in Thinnings 1.719 mg eq/kg		TRR in Forage 0.0148 mg eq/kg		TRR in Stover 0.0644 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Acetone/water extracts	84.4	1.451	67.9	0.0100	66.8	0.0430
Tioxazafen	33.1	0.569	NQ	NA	NQ	NA
Benzoic acid**	NQ	NA	4.2	0.0006	6.5	0.0042
Benzamide	1.9	0.033	4.8	0.0007	4.0	0.0026
Benzamidine	8.8	0.151	12.4	0.0018	11.2	0.0072
Benzoic acid-forming metabolites or conjugates	NQ	NA	17.0	0.0025	14.2	0.0091
Thenoylbenzamidoxime malonylglucoside	7.1	0.122	NQ	NA	NQ	NA
Unknowns (Max. individual)	4.0	0.058	8.3	0.0012	14.4	0.0093
Unextracted (PES)	15.7	0.269	32.0	0.0047	33.3	0.0214
– 0.1 M KOH	7.8	0.133	11.6	0.0017	6.1	0.0039
– 24% KOH	4.6	0.080	15.5	0.0023	17.8	0.0115
Total	100.1	1.72	99.9	0.0147	101.1	0.0644

NQ – Not Quantified (not present or too small to quantify), NA – Not Applicable

** Radioactivity in the HPLC region of benzoic acid was not confirmed by TLC

Table 18 Summary of the Extraction, Characterisation and Identification of the Residues of Tioxazafen in Maize Matrices from the TH Label

Compound	TRR in Thinnings 1.967 mg eq/kg		TRR in Forage 0.0084 mg eq/kg		TRR in Stover 0.0415 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Acetone/water extracts	83.3	1.638	71.0	0.0060	67.8	0.0281
Tioxazafen	46.1	0.907	<1.0	<0.0001	NQ	NA
2-Thiophenecarboxylic acid**	NQ	NA	9.0	0.0008	5.1	0.0021
2-Thiophenecarboxamide**	NQ	NA	2.6	0.0002	3.4	0.0014
Thiophenecarboxylic acid-forming metabolites or conjugates	NQ	NA	12.5	0.0011	11.0	0.0046

Compound	TRR in Thinnings 1.967 mg eq/kg		TRR in Forage 0.0084 mg eq/kg		TRR in Stover 0.0415 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Thenoylbenzamidoxime malonylglucoside	4.2	0.083	NQ	NA	NQ	NA
Unknowns (Max. individual)	4.4	0.087	17.8	0.0015	13.8	0.0057
Unextracted (PES)	16.7	0.33	29	0.0024	32.2	0.0134
– 0.1 M KOH	8.2	0.161	NQ	NA	8.6	0.0036
– 24% KOH	5.9	0.117	NQ	NA	13.5	0.0056
Total	100	1.968	100	0.0084	100	0.0415

NQ – Not Quantified (not present or too small to quantify), NA – Not Applicable

**Radioactivity in the HPLC regions of 2-thiophenecarboxylic acid and 2-thiophenecarboxamide was not confirmed by TLC.

Table 19 Summary of the Extraction of PH-T and TH-T Maize Grain

	TRR of PH-T in Maize Grain 0.0012 mg eq/kg			TRR of TH-T in Maize Grain 0.0020 mg eq/kg		
	% TRR	% TRR normalized	mg eq/kg	% TRR	% TRR normalized	mg eq/kg
Acetone/water extracts	33.6	41.9	0.0005	11.3	11.6	0.0002
Tioxazafen*	NA	NA	<0.0001	NA	NA	<0.0001
Final PES	46.5	58.1	0.0007	86.1	88.4	0.0018

***Maize grain was not analysed by HPLC, but EtOAc partitioning indicates that Tioxazafen is <0.0001 mg/kg.

Metabolism of tioxazafen in maize was extensive leading to a multitude of low-level metabolites. Parent tioxazafen is a major residue only in immature plants (thinnings) harvested about two weeks after emergence and does not exceed 1% of TRR in forage, stover or grain. Partitioning of the concentrated acetone/water extracts of maize grain with ethyl acetate at pH 10 resulted in only a small fraction (5-7%) of the radioactivity partitioning out of the aqueous phase indicating that very little of the residues in the grain are comprised of neutral molecules such as parent tioxazafen. The major metabolite identified in the PH-T maize matrices was benzamidine in thinnings (8.8% TRR, 0.151 mg eq/kg), forage (12.4% TRR, 0.0018 mg eq/kg) and stover (11.2% TRR, 0.0072 mg eq/kg), respectively. Another significant metabolite in the thinnings was thenoylbenzamidoxime malonyl glucoside in the PH-T thinnings (0.122 mg eq/kg, 7.1% TRR) and in the TH-T thinnings (0.083 mg eq/kg, 4.2% TRR). Low residues of benzamide (1.9% TRR, 0.033 mg eq/kg) in thinnings, 4.0-4.8% TRR (0.001-0.003 mg eq/kg) in forage and stover.

All individual metabolite peaks in the HPLC profiles of forage and stover constituted < 0.01 mg eq/kg (each). Other free metabolites observed were trace amounts of benzoic acid, iminoamide, benzamide and thiophene-2-carboxylic acid. Benzoic acid and 2-thiophenecarboxylic acid were also found as conjugates that were cleaved by acid or base treatment of extracts.

No single metabolite exceeded 0.01 mg eq/kg in maize forage, stover or grain. Although a fraction eluting at 25.5-25.8 min in the forage and stover HPLC profiles was integrated as a single peak in the profiles, and exceeded 10% of TRR in some cases, the broad peak eluted in a chromatographic region of unresolved radioactivity and almost certainly represents a mixture of metabolites. Altogether, this fraction did not exceed 0.01 mg eq/kg in any commodity. Although the presence of free benzoic acid and 2-thiophenecarboxylic acid in maize matrices was inconclusive, they both were likely present as conjugates released by hydrolysis.

In summary, parent tioxazafen was a major residual component in thinnings harvested about two weeks after emergence. Parent tioxazafen in thinnings comprised 33.1% TRR (0.569 mg eq/kg) for PH-T and 46.1% TRR (0.907 mg eq/kg) for TH-T, but not exceed 1% TRR in forage, stover or grain. Benzamidine was identified as only major metabolite in thinnings (8.8% TRR, 0.151 mg eq/kg), forage (12.4% TRR, 0.0018 mg eq/kg) and stover (11.2% TRR, 0.0072 mg eq/kg), respectively. Another significant metabolite in the thinnings was thenoylbenzamidoxime malonyl glucoside in the PH-T treatment (7.1% TRR, 0.122 mg eq/kg) and in the TH-T treatment (4.2% TRR, 0.083 mg eq/kg), but not found in forage, stover and grain. Low residues of benzamide were found 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) in PH-T treatment. Other metabolites observed with trace level were benzoic acid, and thiophene-2-carboxylic acid. Benzoic acid and 2-thiophenecarboxylic acid were also found as conjugates that were cleaved by acid or base treatment. No single metabolite exceeded 0.01 mg eq/kg in maize forage, stover or grain.

A proposed pathway for metabolism of tioxazafen in maize plants based on identified/characterised metabolites is presented below in Figure 4. The nature of tioxazafen residues in maize is similar to that in soya bean.

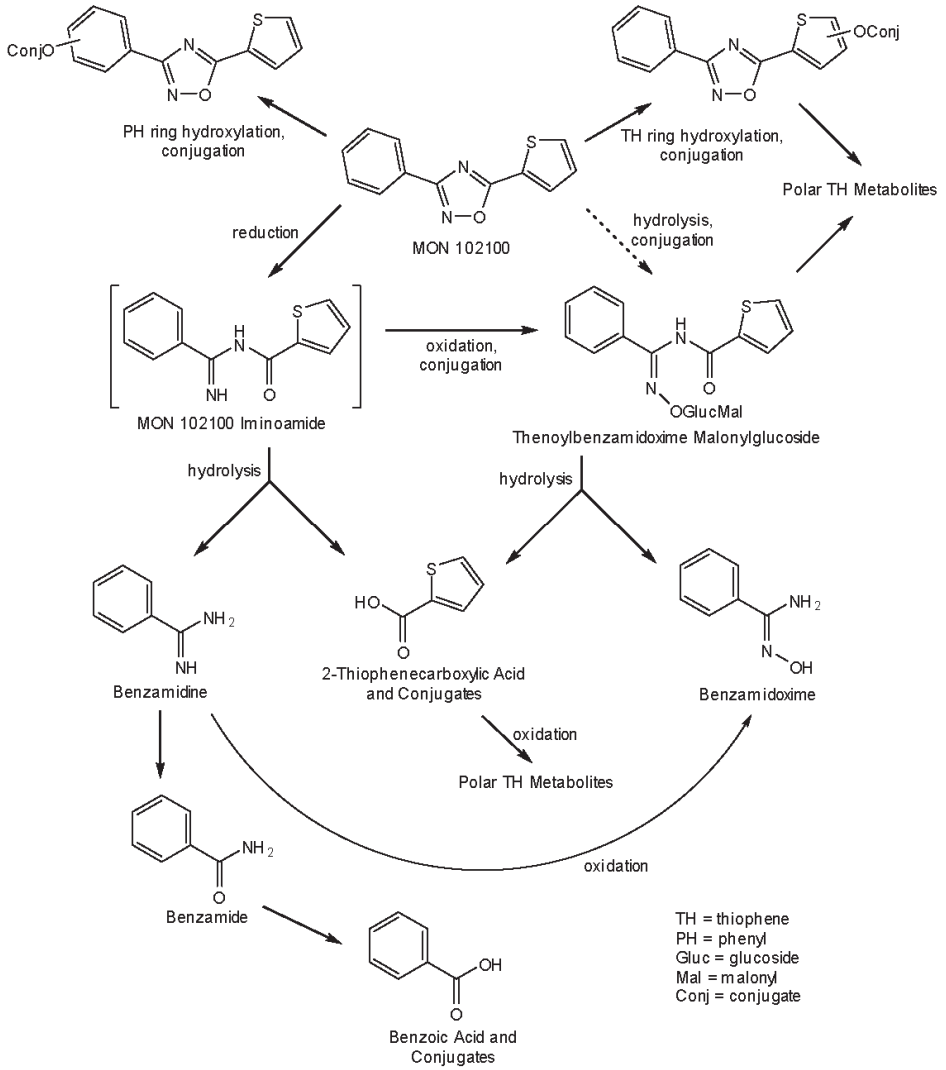


Figure 4 Proposed metabolic pathway for tioxazafen in maize

Cotton (categories of pulses and oilseeds)

A metabolism study was conducted on cotton with two different radiolabelled [^{14}C]tioxazafen to determine the nature of residues in/on cotton, and to identify the pathway of tioxazafen metabolism in cotton following seed treatment (Quistad 2014 MSL0023357).

Pima cotton seeds (*Gossypium barbadense*, PhytoGen PHY 800 PIMA) were treated with [oxadiazole-3-^{13}C, phenyl-UL-^{14}C]-tioxazafen (PH-T) or [oxadiazole-5-^{13}C, thiophene-2-^{14}C]-tioxazafen (TH-T). Each test substance was separately formulated as an aqueous suspension concentrate and applied directly on the seeds using procedures designed to closely mimic intended commercial formulation and seed treatment. The treatment rates were 1.20 mg/seed (0.28 kg ai/ha) for the PH-T treatment and 1.30 mg/seed (0.31 kg ai/ha) for the TH-T treatment.

Treated cotton seeds were planted outdoors in 1.2 m ×1.2 m wooden boxes lined with plastic and filled to a depth of approximately 46 cm with loamy sand soil (pH 7.1, 0.64% organic matter). Immature foliage samples were collected as thinnings 39 days after planting. Leaves/stems and undelinted seed were both collected 182 days after planting. Specimens were frozen promptly after collection, and were maintained frozen through processing (homogenizing) until they were withdrawn for analysis.

Processed cotton matrices were combusted with LSC analysis of the $^{14}CO_2$ trapping solutions. The TRRs were determined from combustion data for thinnings, leaves/stems and delinted seed. Sample processing of undelinted seed separated the seed from the lint. In order to determine an accurate TRR for undelinted seed, the seed and lint were recombined to regenerate "undelinted seed". This mixture was extracted and the homogenous PES) remaining after extraction was combusted. The TRR for undelinted seed was based on the sum of extracts and PES.

Residues of tioxazafen in cotton were low in all matrices as well as similar for both radiolabels in leaves/stems and undelinted seed, whereas thinnings had the highest residue levels. In the thinnings, collected only 39 days after planting, the TRRs were 1.0394 mg eq/kg and 2.4031 mg eq/kg for the PH-T and TH-T specimens, respectively. TRRs for leaves/stems were significantly lower than thinnings with residues of 0.0653 mg eq/kg and 0.0632 mg eq/kg for PH-T and TH-T samples, respectively. The TRR for undelinted seed was determined to be 0.0087 mg eq/kg and 0.0090 mg eq/kg for the PH-T and TH-T samples, respectively. The results of the determination of the total radioactive residue are summarised in Table 20.

Table 20 Distribution of the Residues in Cotton Plant Matrices

Matrix	PHI (days)	PH-T	TH-T	UNT-C
		TRR (mg eq/kg)*	TRR (mg eq/kg)*	TRR (mg eq/kg)*
Thinnings	39	1.0394	2.4031	<0.001
Leaves/Stems	182	0.0653	0.0632	<0.001
Undelinted Seeds**	182	0.0087	0.0090	<0.001

* All TRR values are expressed as Tioxazafen equivalents

** PH-T and TH-T undelinted seed TRR was determined by extraction and combustion of PES

The plant matrices were subjected to exhaustive extraction procedures. Thinnings samples were initially extracted three times with 80:20 (v/v) acetone:water and then one time with 40:60 acetone:water. Leaves and stems were extracted four times with 40:60 acetone:water. Undelinted seed samples (recombined seed and lint) were extracted four times with hexane to extract oils, then once with acetone followed by two extractions with 40:60 acetone:water. For each thinnings and leaves/stems samples, an aliquot of the combined acetone:water extracts was carefully concentrated to remove the acetone prior to HPLC analysis. If the PES was expected to contain >10% of TRR, then further extractions utilised weak base (0.1 M KOH) and strong base (24% KOH). Following extractions, portions (~250 mg) of the PES of thinnings, leaves/stems, and undelinted seeds were combusted to determine the level of unextracted residues.

The PESs obtained from thinnings and leaves/stems extractions were used to investigate the release of unextracted residues through treatment with mild and strong base. Mild base extractions with 0.1 M KOH released substantial residues (11.7-13.0% of TRR) from thinnings from both labels. Mild base extractions of leaves/stems PESs released a smaller amount of residues (2.8-3.0% of TRR) compared to thinnings samples from either label. Strong base extractions with 24% KOH released a substantially larger amount of residues (9.4-16.0% of TRR) from thinnings and leaves/stems.

Four extractions of thinnings with acetone:water (v/v) (three times with 80:20, once with 40:60) extracted 68.9-73.6% of radiolabel (normalized % of TRR). Four extractions of leaves and stems with acetone:water (40:60 (v/v)) extracted 71.2-79.2% of TRR. The post-extraction solids (PES) of thinnings and leaves/stems were further extracted with a weak base (0.1 M KOH) and strong base (24% KOH). Extractions of PES from PH-T and TH-T thinnings with 0.1 M KOH and 24% KOH, extracted 11.7-13.0% and 13.4-16.0% TRR, respectively. Treatment of PES from PH-T and TH-T leaves/stems with 0.1 M KOH and 24% KOH, released 2.8-3.0% and 9.4-12.6% TRR, respectively. Three extractions of undelinted seed (recombined seed and lint) with hexanes, followed by once with acetone, then two times with 40:60 acetone:water extracted 37.9-43.3% TRR. The hexane extracted 13.8-16.6% TRR, while the acetone/water extracts contained 24.1-26.7% TRR; no radioactivity was found in the acetone extracts. Following extractions of all three matrices, portions (~250 mg) of the PES of thinnings, leaves/stems, and undelinted seed were combusted to determine the final level of unextracted radiocarbon.

The results of the extractions and analyses are provided in Table 21 for PH-T and in Table 22 for TH-T. The percent TRR and the mg eq/kg levels were calculated based on the normalized extraction data (sum of the amount extractable and the PES). Because the TRR in the thinnings was much higher than that in the gin byproducts (leaves/stems), the thinnings were analysed in the hope that the higher TRR would provide some insight into the early metabolism of tioxazafen and would facilitate the identification of metabolites.

Tioxazafen is extensively metabolized in cotton to a complex mixture of residues. Parent tioxazafen is observed as a residue only in thinnings harvested 39 days after planting. No single metabolite exceeded 0.01 mg eq/kg in the raw agricultural commodities gin-byproducts (analysed as leaves/stems) or undelinted seed. Benzamidine was the only major metabolite observed, present at low levels (<0.01 mg eq/kg) but representing 10.9% TRR in leaves/stems and 6.2% TRR in

thinnings. A small amount of benzamide was also present in thinnings and leaves/stems (4.0-7.6% TRR). Low levels of benzoic acid and 2-thiophenecarboxylic acid (each <10% TRR) appeared to be present in the HPLC profiles of the foliage matrices, but confirmation by partitioning and/or TLC was not definitive. It is likely that benzoic acid and 2-thiophenecarboxylic acid were present in these matrices as conjugates based on the results of hydrolysis experiments. The large amounts of unextracted radioactivity in the PH-T and TH-T undelinted seed, and significant radioactivity in the hexane extract of the oil fraction (that did not partition out of hexane with acetonitrile), is consistent with incorporation of small molecules, derived through extensive metabolism of tioxazafen, into natural products (e.g., proteins, triglycerides).

Table 21 Summary of the Extraction, Characterisation and Identification of the Residues of Tioxazafen in Cotton Matrices from the PH Label

Compound	Thinnings TRR = 1.0394 mg eq/kg		Leaves and Stems TRR = 0.0653 mg eq/kg		Undelinted Seed[b] TRR = 0.0087 mg eq/kg	
	% TRR	mg eq/kg[f]	% TRR	mg eq/kg[f]	%TRR	mg eq/kg[f]
Combined acetone/water extracts	73.6	0.765	79.2	0.0517	24.1	0.0021
Hexane extract	NA	NA	NA	NA	13.8	0.0012
Benzamidine	6.2	0.064	10.9	0.0071	NQ	NA
Benzoic acid[a]	7.5	0.078	3.7	0.0024	NQ	NA
Iminoamide[a]	NQ	NA	12.5	0.0082	NQ	NA
Benzamide	4.0	0.042	7.6	0.0050	NQ	NA
Maximum other single[e]	11.0	0.114	10.9	0.0071	NQ	NA
Tioxazafen	6.3	0.065	NQ	NQ	NQ	NA
0.1 M KOH	11.7	0.122	2.8	0.0018	NA	NA
24% KOH	13.4	0.139	9.4	0.0061	NA	NA
Total Extracted	98.7	1.026	91.4	0.0597	37.9	0.0033
Final Post-Extraction Solid	1.3	0.013	8.6	0.0056	62.1	0.0054

NQ – Not Quantified (not present or too small to quantify), NA – Not Applicable

[a] Radioactivity in HPLC regions of benzoic acid and Iminoamide were not confirmed by TLC.

[b] Extracts of undelinted seed were not analysed by HPLC, but hexane/acetonitrile partitioning indicates Tioxazafen represents <0.001 mg/kg.

[c] The TRR presented for thinnings and leaves/stems was determined by combustion; the TRR for undelinted seed represents the sum of the extractable and PES radioactivity. Totals may not equal the sum of each value due to rounding.

[d] Represents the sum of benzamidine residues in the analysis of the extract and the amount quantified after isolation of a polar peak at 4 min in the HPLC of the extract.

[e] Maximum single peak not identified.

[f] mg eq/kg values are expressed as Tioxazafen equivalents.

Table 22 Summary of the Extraction, Characterisation and Identification of the Residues of Tioxazafen in Cotton Matrices from the TH Label

Compound	Thinnings TRR = 2.4031 mg eq/kg		Leaves and Stems TRR = 0.0632 mg eq/kg		Undelinted Seed[b] TRR = 0.0090 mg eq/kg	
	% TRR	mg eq/kg[f]	% TRR	mg eq/kg[f]	%TRR	mg eq/kg[f]
Combined acetone/water extracts	68.9	1.656	71.2	0.0450	26.7	0.0024
Hexane extracts	NA	NA	NA	NA	16.6	0.0015
2-Thiophenecarboxylic acid[a]	9.4	0.226	7.9	0.0050	NQ	NA
2-Thiophenecarboxamide[a]	0.8	0.019	NQ	NA	NQ	NA
Maximum other single[e]	6.5	0.156	19.4	0.0123	NQ	NA
Tioxazafen	15.7	0.377	NQ	NA	NQ	NA
0.1 M KOH	13.0	0.311	3.0	0.0019	NA	NA
24% KOH	16.0	0.385	12.6	0.0080	NA	NA
Total Extracted	97.9	2.352	86.8	0.0549	43.3	0.0039
Final Post-Extraction Solid	2.1	0.051	13.3	0.0084	56.7	0.0051

NQ – Not Quantified (not present or too small to quantify); NA – Not Applicable

[a] Radioactivity in HPLC regions of 2-thiophenecarboxylic acid and 2-thiophenecarboxamide were not confirmed by TLC.

[b] Extracts of undelinted seed were not analysed by HPLC, but hexane/acetonitrile partitioning indicates Tioxazafen represents <0.001 mg/kg.

[c] The TRR presented for thinnings and leaves/stems was determined by combustion; the TRR for undelinted seed represents the sum of the extractable and PES radioactivity. Totals may not equal the sum of each value due to rounding.

[d] Represents the sum of benzamidine residues in the analysis of the extract and the amount quantified after isolation of a polar peak at 4 min in the HPLC of the extract.

[e] Maximum single peak not identified.

[f] mg eq/kg values are expressed as Tioxazafen equivalents.

Metabolism of tioxazafen in cotton was extensive, leading to a multitude of low-level metabolites. The major ^{14}C-residues in PH-T thinnings were tioxazafen (6.3% TRR, 0.065 mg eq/kg), while the major metabolites identified were benzamidine (6.2% TRR, 0.064 mg eq/kg), benzoic acid (7.5% TRR, 0.078 mg eq/kg, not identified by TLC) and benzamide (4.0% TRR, 0.042 mg eq/kg). No other single metabolite or chromatographic region represented >6% TRR. The major ^{14}C-residue in TH-T thinnings was Tioxazafen (15.7% TRR, 0.377 mg eq/kg), the major metabolites identified were 2-thiophenecarboxylic acid (9.4% TRR, 0.226 mg eq/kg) and 2-thiophenecarboxamide (0.8% TRR, 0.019 mg eq/kg). No other single metabolite represented >7% of TRR.

In PH-T leaves/stems, tioxazafen was not detected, and all individual metabolite residues were <0.01 mg eq/kg with low-level peaks eluting in the regions corresponding to benzoic acid (3.7% TRR, 0.0024 mg eq/kg, not identified by TLC), benzamide (7.6% of TRR, 0.0050 mg eq/kg), and benzamidine (10.9% TRR, 0.0071 mg eq/kg). An early-eluting HPLC region (0-15 min) was isolated and reanalysed using a different HPLC method. The reanalysis gave benzamidine as the major component (63.6% of the profile) and a small amount of benzamidoxime (3.3% of the profile). As seen in PH-T, tioxazafen was not detected in TH-T leaves/stems. All residues in TH-T leaves/stems were <0.01 mg eq/kg with a low-level peak of radioactivity eluting in the region consistent with 2-thiophenecarboxylic acid (7.9% TRR, 0.0050 mg eq/kg). No other single metabolite represented >0.01 mg eq/kg.

To release aglycones from possible conjugates and hydrolyze metabolites to their respective chemophores (e.g., benzoic acid or 2-thiophenecarboxylic acid) in PH-T and TH-T leaves/stems, a base hydrolysis of the combined, concentrated acetone:water extracts was employed. The HPLC analysis of the PH-T leaves/stems NaOH hydrolysates gave two major peaks, benzoic acid (27.1% TRR, 0.0142 mg eq/kg) and a peak eluting in the benzamidine/benzamidoxime region (22.7% TRR, 0.0149 mg eq/kg) likely arising from partial hydrolysis to benzoic acid. As observed in the acid and base hydrolysates of maize stover extracts in the tioxazafen maize metabolism study, minor peaks at 19.6 and 21.6 min, consistent with the retention times of 4-hydroxybenzoic acid and 3-hydroxybenzoic acid, respectively, were observed in the HPLC profile of the PH-T leaves/stems NaOH hydrolysates. For the TH-T leaves/stems, HPLC analysis of the NaOH hydrolysates gave a small amount of 2-thiophenecarboxylic acid (3.5% TRR, 0.0022 mg eq/kg). The majority of the radioactivity in the hydrolysate was of a polar, early-eluting in nature. The small amount of 2-thiophenecarboxylic acid produced by hydrolysis, coupled with the polar nature of the hydrolysis products, indicates that the majority of the cotton leaves/stems metabolites are functionalized on the thiophene ring.

Due to the low-level of radioactive residues in PH-T and TH-T undelinted seed, metabolites were not identified and/or characterised. To determine the presence of tioxazafen, the hexane extracts from PH-T and TH-T undelinted seed were separately partitioned with acetonitrile. The majority of the radioactive residues (>95% of TRR) from both PH-T and TH-T seed remained in the hexane phase. As observed in the tioxazafen maize and soya bean metabolism, tioxazafen is expected to be extracted by hexane with the oil fractions of seeds, but a tioxazafen reference standard was shown to entirely partition into the acetonitrile phase when partitioned between hexane and acetonitrile. Thus, the seed extract partitioning results indicate that there is very little or virtually no tioxazafen, or non-conjugated metabolites of similar polarity to tioxazafen, in PH-T or TH-T undelinted seed. The large amounts of unextracted radioactivity in the PH-T and TH-T undelinted seed, and significant radioactivity in the oil fraction (hexane extract) that did not partition out of hexane, are consistent with incorporation of small molecules, derived through extensive metabolism of tioxazafen, into natural products (e.g., proteins, triglycerides).

A proposed pathway for metabolism of Tioxazafen in cotton plants based on identified/characterised metabolites is present below in Figure 5.

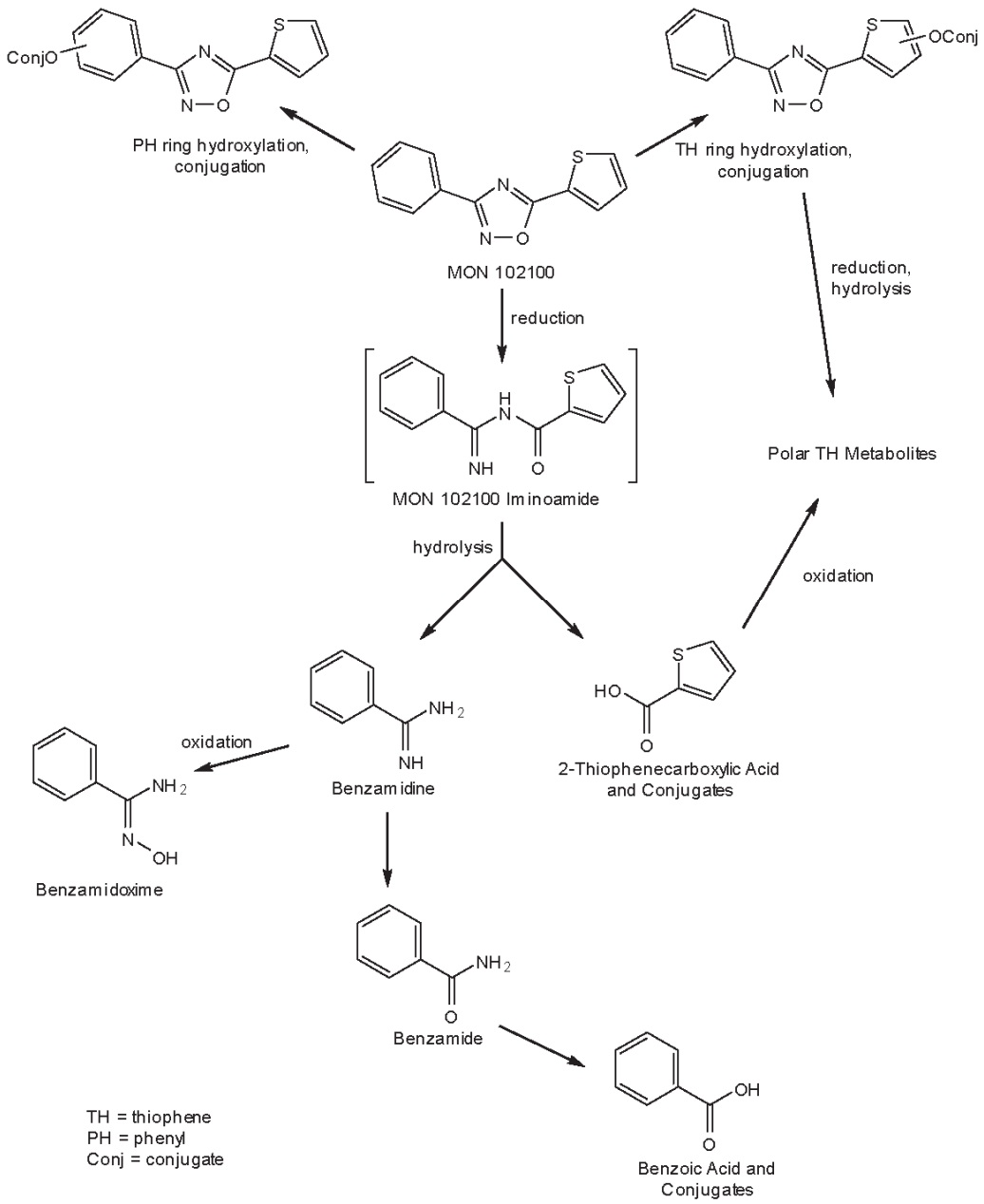


Figure 5 Proposed Metabolic Pathway for Tioxazafen in Cotton

In summary for plants, a major pathway for metabolism of tioxazafen is cleavage of the oxadiazole ring. The initial metabolite is likely tioxazafen iminoamide formed by reductive cleavage of the N-O bond of the oxadiazole ring, although this metabolite is likely transient and is not observed in any cotton matrix. The identified metabolites that are formed from tioxazafen, especially benzamidine, implicate the iminoamide as the likely intermediate. The iminoamide metabolite of tioxazafen has been observed in other systems in which benzamidine has been observed as a major metabolite. Iminoamide intermediates have also been observed in the metabolism of other 1,2,4-oxadiazoles.

Hydrolysis of the iminoamide intermediate, which is likely enzyme-mediated, leads to benzamidine and 2-thiophenecarboxylic acid. Benzamidine is a significant residue representing up to 11% TRR in thinnings and leaves/stems (surrogate for gin by-products). Hydrolysis of benzamidine leads to benzamide and further to benzoic acid, both of which represented <10% TRR in thinnings and leaves/stems. Although benzoic acid and 2-thiophenecarboxylic acid were not

confirmed as free metabolites, they are likely present as conjugate(s) based on formation of benzoic acid and 2-thiophenecarboxylic acid on hydrolysis of the PH-T and TH-T extracts, respectively.

As additional potential pathways of metabolism, hydroxylation (and subsequent conjugation) of the phenyl ring and the thiophene ring of Tioxazafen likely to occur. Acid or base hydrolysis reactions of PH-T foliage extracts formed minor amounts of hydrolysis products with HPLC retention times consistent with those of 3- and 4-hydroxybenzoic acids providing evidence for hydroxylation of the phenyl ring of tioxazafen. Overall, however, hydroxylation of the phenyl ring is considered a minor pathway and the majority of residues appeared to contain an unsubstituted phenyl ring. Oxidation of the thiophene ring of tioxazafen or its metabolites is likely a significant metabolic pathway in plants. Although no specific metabolites with a functionalized thiophene ring were identified in plants, the thiophene ring is known to be susceptible to oxidation, and conjugates (sulfate, glucuronide or malonylglucoside) of metabolites hydroxylated on the thiophene ring of tioxazafen have been identified in both animal (goat, hen and rat) and plant (soya bean) metabolism studies of tioxazafen.

Rotational crop studies

Confined rotational crop studies

The meeting received information on a confined rotational crop study.

Maize seeds treated with phenyl or thiophene-^{14}C labelled tioxazafen at rate of 0.5 mg/seed, (equivalent to 0.32 kg ai/ha) were planted in sandy loam soil (pH 5.9, 0.82% organic matter) in 1 m^2 wooden boxes lined with plastic. Maize seedlings were cut off near the soil surface at approximately two weeks after emergence, and were roughly chopped and tilled into the soil.

Three rotational crops: leaf lettuce (*Lactuca sativa*, representing the leafy vegetables), radish (*Raphanus sativus*, representing root crops), and wheat (*Triticum aestivum*, representing small grain crops) were grown in soil as rotational crops at intervals of 30, 120 and 360 days after the maize was planted. The original 360-day lettuce crop failed, and a replacement crop was planted at 413 days.

Immature lettuce was harvested at approximately half-size for commercial harvest, all remaining lettuce was harvested at maturity. The tops (foliage) 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.

All samples were processed in the presence of dry ice, and the TRR of each was determined by combustion. Crop samples from all 30-day samples or samples of the TRR above 0.01 mg eq/kg were extracted with acetone: water (40:60, 100 mL, 2-4×). The extracts were concentrated and analysed by reversed phase HPLC to characterise the metabolites. TLC was also used for characterisation, when feasible. The post-extraction solids (PES) were then sequentially treated with 0.1 M KOH and 4 M KOH, if the PES residue warranted further examination. KOH extracts containing >10% TRR were acidified to pH 2-3 and partitioned with ethyl acetate. The organic and aqueous phases were quantified by LSC.

Only trace amounts of tioxazafen were found in the raw agricultural commodities analysed in this study. Because of the extremely low levels of these metabolites, assignment of their identity was made via retention time correlation; confirmation by a second method was not feasible. Very low levels (≤0.006 mg eq/kg) of benzamidine, benzamide, benzoic acid, and tioxazafen iminoamide were found in several crop samples from the PH-label. In the TH-labelled commodities, low levels of tioxazafen imide and iminoamide and 2-thiophenecarboxylic acid were detected. Except for 2-thiophenecarboxylic acid, which reached up to 0.007 mg eq/kg, the levels of the metabolites in rotation crops from the TH plots were ≤0.006 mg eq/kg, and were generally well below 10% TRR. The only other metabolite which was found at or above 10% TRR was an unknown metabolite which was prominent in wheat forage, hay and straw samples, with a retention time 18.3-18.8 min. This metabolite appeared in both PH and TH samples and, in some samples, approached 22% TRR, but its concentration only reached a maximum of 0.008 mg eq/kg, too low to permit identification.

The TRR was generally maximum at the 120-day interval for both radiolabels. The TRR are provided in Table 23.

Table 23 Total Radioactive Residues in RACs as Determined by Combustion

Matrix	PBI (days)	Phenyl-^{14}C	Thiophene-^{14}C
		TRR (mg equiv./kg)	TRR (mg equiv./kg)
Immature lettuce	30	0.0083	0.0072
	120	0.0075	0.0095
	413	0.0038	0.0025
Mature lettuce	30	0.0041	0.0048
	120	0.0043	0.0071
	413	0.0023	0.0030
Radish foliage	30	0.0049	0.0058

Matrix	PBI (days)	Phenyl-^{14}C	Thiophene-^{14}C
		TRR (mg equiv./kg)	TRR (mg equiv./kg)
	120	0.0104	0.0184
	360	0.0014	0.0050
Radish root	30	0.0073	0.0096
	120	0.0503	0.0568
	360	0.0054	0.0145
Wheat forage	30	0.0157	0.0160
	120	0.0422	0.0226
	360	0.0148	0.0242
Wheat hay	30	0.0315	0.0552
	120	0.0713	0.0526
	360	0.0228	0.0339
Wheat straw	30	0.0435	0.0592
	120	0.0769	0.0869
	360	0.0176	0.0220
Wheat grain	30	0.0039	0.0035
	120	0.0070	0.0050
	360	0.0029	0.0032

Each of the RACs from the 30-Day planting was extracted and analysed regardless of the TRR of the samples. Samples from later plantings were not extracted and analysed when the TRR was considerably below the 0.01 mg eq/kg criterion for further analysis. The extractability and the distribution of the radioactivity in the metabolites were based on the normalized percent TRR.

Residues in Immature Lettuce Rotational Crops**:** The TRRs in immature lettuce were low from all three plantings in both labels; the TRRs of the 360-day specimens were so low that they were not analysed. Tioxazafen was found at 0.0003 mg eq/kg in the 120-day PH and 0.0001 mg eq/kg in the 30-day TH immature lettuce, but in no other samples. Benzamidine represented <5% TRR in the 30-day and 120-day PH immature lettuce. Summaries of the characterisation of the TRR in immature lettuce are provided in Table 24.

Table 24 Summary of Characterisation and Identification of Radioactive Residues in Immature Lettuce Following Seed-Treatment Application of [Phenyl-^{14}C] Tioxazafen

	Immature Lettuce – 30 day TRR 0.0083 mg eq/kg		Immature Lettuce – 120 day TRR 0.0075 mg eq/kg		Immature Lettuce – 413 day TRR 0.0038 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	4.4	0.0003	NA	NA
Benzoic acid	1.7	0.0001	ND	NA	NA	NA
Tioxazafen Imide	ND	NA	2.4	0.0002	NA	NA
RT 18.3 - 18.8 min	4.0	0.0003	ND	NA	NA	NA
Benzamidine	2.2	0.0002	3.6	0.0003	NA	NA
Maximum other single†	24.0	0.0020	13.4	0.0010	NA	NA
Total identified	3.9	0.0003	10.4	0.0008	NA	NA
Total characterised[1]	77.8	0.0065	63.7	0.0048	NA	NA
Total extracted	81.7	0.0068	74.1	0.0056	NA	NA
Unextracted (PES)*	18.3	0.0016	25.9	0.0019	NA	NA
Released by harsh treatments	4.3#	0.0004	NA	NA	NA	NA
Accountability**	97.2		114.9		NA	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed.

0.1 M KOH

[1]Other characterised included RT 26.8, 23.3, 22.8, 20.8, 3.8min from 30PBI, RT 26.8, 24.8, 22.8, 19.8, 17.8, 4.3 min from 120PBI

Table 25 Summary of Characterisation and Identification of Radioactive Residues in Immature Lettuce Following Seed-Treatment Application of [Thiophene-^{14}C]Tioxazafen

	Immature Lettuce – 30 day TRR 0.0072 mg eq/kg		Immature Lettuce – 120 day TRR 0.0095 mg eq/kg		Immature Lettuce – 413 day TRR 0.0025 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	0.7	0.0001	ND	NA	NA	NA
2-Thiophenecarboxamide	ND	NA	7.0	0.0007	NA	NA
Maximum other single†	15.6	0.0011	12.9	0.0012	NA	NA
Total identified	0.7	0.0001	7.0	0.0007	NA	NA
Total characterised	76.8	0.0055	71.7	0.0068	NA	NA
Total extracted	77.5	0.0056	78.7	0.0074	NA	NA
Unextracted (PES)*	22.5	0.0016	21.3	0.0020	NA	NA
Released by harsh treatments	4.4#	0.0003	NA	NA	NA	NA
Accountability**	92.7		100.1		NA	NA

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

0.1 M KOH

Residues in Mature Lettuce Rotational Crops:

The TRRs in mature lettuce were all well below the 0.01 mg eq/kg level requiring further analysis; however, the 30-day and 120-day samples from both labels were extracted in order to characterise the radioactive residues. Traces of Tioxazafen (<0.0001 mg eq/kg) were found in the 30-day TH and the 120-day PH and TH samples. No residues were identified at >6% TRR, or detected at >16% TRR. The analyses are summarised in Table 26 and Table 27.

Table 26 Summary of Characterisation and Identification of Radioactive Residues in Mature Lettuce Following Seed-Treatment Application of [Phenyl-^{14}C]Tioxazafen

	Mature Lettuce –		Mature Lettuce –		Mature Lettuce –	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	0.7	<0.0001	NA	NA
Benzoic acid	ND	NA	5.0	0.0002	NA	NA
Tioxazafen Imide	ND	NA	0.9	<0.0001	NA	NA
Benzamide	ND	NA	ND	NA	NA	NA
RT 18.3 - 18.8	5.2	0.0002	6.0	0.0003	NA	NA
Benzamidine	3.8	0.0002	2.4	0.0001	NA	NA
Maximum other single†	14.2	0.0006	15.2	0.0007	NA	NA
Total identified	3.8	0.0002	9.0	0.0005	NA	NA
Total characterised	72.3	0.0030	63.2	0.0027	NA	NA
Total extracted	76.1	0.0031	72.2	0.0031	NA	NA
Unextracted (PES)*	23.8	0.0010	27.8	0.0012	NA	NA
Released by harsh treatments	NA	NA	16.9#	0.0007	NA	NA
Accountability**	99.5		134.2		NA	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

0.1 M KOH

Table 27 Summary of Characterisation and Identification of Radioactive Residues in Mature Lettuce Following Seed-Treatment Application of [Thiophene-^{14}C]Tioxazafen

	Mature Lettuce – 30 day TRR 0.0048 mg eq/kg		Mature Lettuce – 120 day TRR 0.0071 mg eq/kg		Mature Lettuce – 413 day TRR 0.0030 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	0.2	<0.0001	0.7	<0.0001	NA	NA
2-Thiophenecarboxylic acid	ND	NA	5.7	0.0004	NA	NA
2-Thiophenecarboxamide	ND	NA	0.5	<0.0001	NA	NA
RT 18.3 - 19.3	3.8	0.0002	5.5	0.0004	NA	NA
Maximum other single†	12.8	0.0006	12.2	0.0009	NA	NA
Total identified	0.2	<0.0001	6.9	0.0005	NA	NA
Total characterised	77.7	0.0037	66.5	0.0047	NA	NA
Total extracted	77.9	0.0037	73.4	0.0052	NA	NA
Unextracted (PES)*	22.1	0.0011	26.7	0.0019	NA	NA
Released by harsh treatments	NA	NA	14.9#	0.0011	NA	NA
Accountability**	99.2		125.9		NA	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

0.1 M KOH

Residues in Radish Foliage:

The TRRs in radish foliage exceeded 0.01 mg eq/kg only in the 120-day samples from both labels. Tioxazafen was detected in only the 120-day PH-T radish foliage, at <0.0001 mg eq/kg. Very low levels of several minor metabolites were seen in the PH-T foliage, but none exceeded 0.001 mg eq/kg. In the TH-T radish foliage, 2-thiophenecarboxylic acid represented 10.1% TRR (0.0006 mg eq/kg) in the 30-day TH-T radish foliage and 16.2% TRR (0.0030 mg eq/kg) in the 120-day TH-T radish foliage. Other TH-T metabolites did not exceed 0.003 mg eq/kg. Summaries of the distribution of the TRR in radish foliage may be found in Tables 28 and 29.

Table 28 Summary of Characterisation and Identification of Radioactive Residues in Radish Foliage Following Seed-Treatment Application of [Phenyl-^{14}C]Tioxazafen

	Radish Foliage – 30 day TRR 0.0049 mg eq/kg		Radish Foliage – 120 day TRR 0.0104 mg eq/kg		Radish Foliage – 360 day TRR 0.0014 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	0.2	<0.0001	NA	NA
Benzoic acid region	5.2	0.0003	3.0	0.0003	NA	NA
Tioxazafen Imide	0.6	<0.0001	1.1	0.0001	NA	NA
Tioxazafen Iminoamide	4.7	0.0002	3.8	0.0004	NA	NA
Benzamide	2.9	0.0001	4.0	0.0004	NA	NA
RT 18.3 - 18.8	5.8	0.0003	3.7	0.0004	NA	NA
Benzamidine	2.2	0.0001	1.1	0.0001	NA	NA
Maximum other single†	21.1	0.0010	9.1	0.0009	NA	NA
Total identified	15.6	0.0008	13.2	0.0014	NA	NA
Total characterised	76.2	0.0037	73.7	0.0077	NA	NA
Total extracted	91.8	0.0045	86.9	0.0090	NA	NA
Unextracted(PES)*	8.2	0.0004	13.1	0.0014	NA	NA
Released by harsh treatments	NA	NA	4.4#	0.0005	NA	NA
Accountability**	108.0		100.0		NA	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

0.1 M KOH

Table 29 Summary of Characterisation and Identification of Radioactive Residues in Radish Foliage Following Seed-Treatment Application of [Thiophene- ^{14}C]Tioxazafen

	Radish Foliage – 30 day TRR =		Radish Foliage – 120 day TRR =		Radish Foliage – 360 day TRR =	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	ND	NA	NA	NA
2-Thiophenecarboxylic acid	10.1	0.0006	16.2	0.0030	NA	NA
Tioxazafen Imide	0.9	0.0001	1.5	0.0003	NA	NA
2-Thiophenecarboxamide	3.6	0.0002	1.1	0.0002	NA	NA
RT 18.3 - 18.8	3.8	0.0002	6.0	0.0011	NA	NA
Maximum other single†	16.9	0.0010	15.7	0.0029	NA	NA
Total identified	14.6	0.0009	18.8	0.0035	NA	NA
Total characterised	74.2	0.0043	68.7	0.0126	NA	NA
Total extracted	88.8	0.0052	87.5	0.0161	NA	NA
Unextracted(PES)*	11.2	0.0006	12.5	0.0023	NA	NA
Released by harsh treatments	NA	NA	4.3#	0.0008	NA	NA
Accountability**	111.9		98.2		NA	NA

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

0.1 M KOH

Residues in Radish Roots

Residues in radish roots were highest in samples from the 120-day planting; the TRRs were 0.0503 mg eq/kg (PH-T) and 0.0568 mg eq/kg (TH-T). TRRs at the other sampling times were less than 0.01 mg eq/kg for the PH-T treatment and were 0.0096 mg eq/kg (30-day planting) and 0.0145 mg eq/kg (360-day planting for the TH-T treatment). Tioxazafen was not detected in any radish root sample. In the 30-day PH-T radish root, no metabolites were observed above 0.002 mg eq/kg; one unidentified metabolite was 15.4% TRR, but only 0.0011 mg eq/kg. Numerous metabolites were found in the PH-T samples but only a metabolite in the benzoic acid region at 10.9% TRR, an unidentified metabolite (RT 18.3-18.8) at 9.0% TRR (0.0045 mg eq/kg) and another unidentified metabolite at 9.9% (0.005 mg eq/kg) were significant in the 120-day PH-T radish root; all were well below 0.01 mg eq/kg. In the TH-T samples, 2-thiophenecarboxylic acid was found at 9% and 11.8% TRR in the 30-day and 120-day TH-T samples, respectively. The only other metabolite of note seen in radish root was an unidentified metabolite with a retention time of 18.3-18.8, which may be related to a metabolite of similar retention time in wheat forage, hay and straw. In radish root, this metabolite represented 5.6 and 8.9% TRR in the 30-day and 120-day TH-T radish root, and 6.7% TRR in the 30-day PH-T radish root. As shown in Tables 30 and 31, none of the metabolites were ≥ 0.01 mg eq/kg.

Table 30 Summary of Characterisation and Identification of Radioactive Residues in Radish Roots after Seed-Treatment Application of [Phenyl-^{14}C]Tioxazafen

	Radish Roots – 30 day TRR 0.0073 mg eq/kg		Radish Roots – 120 day TRR 0.0503 mg eq/kg		Radish Roots – 360 day TRR 0.0054 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	ND	NA	NA	NA
Benzoic acid region	6.7	0.0005	10.9	0.0055	NA	NA
Tioxazafen Imide	ND	NA	2.7	0.0014	NA	NA

	Radish Roots – 30 day TRR 0.0073 mg eq/kg		Radish Roots – 120 day TRR 0.0503 mg eq/kg		Radish Roots – 360 day TRR 0.0054 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen Iminoamide	6.0	0.0004	5.6	0.0028	NA	NA
Benzamide	ND	NA	ND	NA	NA	NA
RT 18.3 - 18.8	6.7	0.0005	9.0	0.0045	NA	NA
Benzamidine	2.9	0.0002	2.2	0.0011	NA	NA
Maximum other single†	15.4	0.0011	9.9	0.0050	NA	NA
Total identified	15.6	0.0011	21.4	0.0108	NA	NA
Total characterised	49.3	0.0036	61.6	0.0310	NA	NA
Total extracted	64.9	0.0047	83.0	0.0417	NA	NA
Unextracted(PES)*	35.1	0.0026	17.0	0.0085	NA	NA
Released by harsh treatments	NA	NA	13.0#	0.0065	NA	NA
Accountability**	117.4		120.5		NA	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

0.1 M KOH

Table 31 Summary of Characterisation and Identification of Radioactive Residues in Radish Roots after Seed-Treatment Application of [Thiophene-^{14}C]Tioxazafen

	Radish Roots – 30 day TRR 0.0096 mg eq/kg		Radish Roots – 120 day TRR 0.0568 mg eq/kg		Radish Roots – 360 day TRR 0.0145 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	ND	NA	ND	NA
2-Thiophenecarboxylic acid	9.0	0.0009	11.8	0.0067	5.4	0.0008
Tioxazafen Imide	ND	NA	3.7	0.0021	ND	NA
Tioxazafen Iminoamide	ND	NA	6.1	0.0035	ND	NA
2-Thiophenecarboxamide	1.1	0.0001	ND	NA	ND	NA
RT 18.3-18.8	5.6	0.0005	8.9	0.0051	NA	NA
Maximum other single†	9.8	0.0009	12.0	0.0068	11.9	0.0017
Total identified	10.1	0.0010	21.6	0.0123	5.4	0.0008
Total characterised	52.8	0.0050	59.1	0.0335	54.3	0.0079
Total extracted	62.9	0.0060	80.7	0.0458	59.7	0.0087
Unextracted(PES)*	37.1	0.0036	19.3	0.0109	34.2	0.0059
Released by harsh treatments	NA	NA	15.2#	0.0086	33.6##	0.0049
Accountability**	118.5		89.6		108.5	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

0.1 M KOH (7.8% TRR, 0.0044 mg eq/kg) followed by 24% KOH (7.4% TRR, 0.0042 mg eq/kg)

0.1 M KOH (5.6% TRR, 0.0008 mg eq/kg) followed by 24% KOH (28.0% TRR, 0.0041 mg eq/kg)

Residues in wheat forage:

The TRRs in wheat forage ranged from 0.015-0.042 mg eq/kg in samples from 30-, 120- and 360-day plantings in both the PH-T and TH-T treatments. In wheat, the major metabolite of note was an unidentified metabolite eluting at 18.3-19.3 min. While the peak retention time varied slightly, possibly due to the amount of matrix-related material in the sample analysed, it was a fairly sharp peak. This unknown was found in both treatment groups at 17.4-22% TRR. There were other unknown metabolites

in wheat forage, but none exceeded 0.007 mg eq/kg. Tioxazafen was detected only in the 120-day wheat forage from the PH-T group. Other metabolites were found in both treatment groups, usually well below 10% TRR. A summary of the characterisation of the residues in wheat forage may be found in Tables 32 33.

Table 32 Summary of Characterisation and Identification of Radioactive Residues in Wheat Forage Following Seed-Treatment Application of [Phenyl-^{14}C]Tioxazafen

	Wheat Forage – 30 day TRR 0.0157 mg eq/kg		Wheat Forage – 120 day TRR 0.0422 mg eq/kg		Wheat Forage – 360 day TRR 0.0148 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	2.5	0.0011	ND	NA
Benzoic acid	3.3	0.0005	3.9	0.0016	8.8	0.0013
Tioxazafen Imide	0.4	0.0001	0.6	0.0003	ND	NA
Tioxazafen Iminoamide	ND	NA	9.4	0.0040	ND	NA
Benzamide	2.8	0.0004	3.2	0.0014	ND	NA
RT 18.3-19.3	18.9	0.0030	19.6	0.0083	17.4	0.0026
Benzamidine	3.0	0.0005	1.2	0.0005	6.7	0.0010
Maximum other single†	20.4	0.0032	16.4	0.0069	11.2	0.0017
Total identified	9.5	0.0015	20.8	0.0089	15.5	0.0023
Total characterised	73.2	0.0115	67.9	0.0285	64.4	0.0095
Total extracted	85.7	0.0135	88.7	0.0374	79.9	0.0118
Unextracted (PES)*	14.3	0.0022	11.3	0.0048	20.1	0.0030
Released by harsh treatments #	7.7	0.0012	6.3	0.0027	7.6	0.0011
Accountability**	108.0		98.6		99.5	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

0.1 M KOH

Table 33 Summary of Characterisation and Identification of Radioactive Residues in Wheat Forage Following Seed-Treatment Application of [Thiophene-^{14}C]Tioxazafen

	Wheat Forage – 30 day TRR 0.0160 mg eq/kg		Wheat Forage – 120 day TRR 0.0226 mg eq/kg		Wheat Forage – 360 day TRR 0.0242 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	ND	NA	ND	NA
2-Thiophenecarboxylic acid	10.9	0.0017	10.4	0.0024	7.6	0.0018
Tioxazafen Imide	0.7	0.0001	0.5	0.0001	ND	NA
Tioxazafen Iminoamide	3.8	0.0006	4.5	0.0010	ND	NA
2-Thiophenecarboxamide	4.2	0.0007	ND	NA	ND	NA
RT 18.3-19.3	21.7	0.0035	21.2	0.0048	22.0	0.0053
Maximum other single†	7.7	0.0012	8.5	0.0019	10.0	0.0024
Total identified	19.6	0.0031	15.4	0.0035	7.6	0.0018
Total characterised	59.1	0.0095	69.9	0.0158	70.3	0.0170
Total extracted	78.7	0.0126	85.3	0.0193	77.9	0.0189
Unextracted (PES)*	21.2	0.0034	14.7	0.0033	22.1	0.0054
Released by harsh treatments #	9.0	0.0014	8.4	0.0019	8.2	0.0020
Accountability**	80.9		90.4		92.3	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

0.1 M KOH

Residues in Wheat Hay:

The TRR in wheat hay ranged from 0.023 to 0.071 mg eq/kg in the sampling from both the PH-T and TH-T groups at all three planting intervals. The unknown metabolite eluting at 18.3-18.8 min was the most abundant metabolite, representing 14% TRR and 14.8% TRR in the PH-T and TH-T from the 30-day planting. In the samples from the 120-day planting, it amounted to 8.3% TRR and 12.1% TRR in the PH-T and TH-T treatments, respectively. The same metabolite represented 5-6% TRR in the samples from the 360-day planting. Other unidentified metabolites represented <0.01 mg eq/kg. Benzamidine was present at ≤5% in the PH-T wheat hay and 2-thiophenecarboxylic acid was present at 5-7% in the TH-T wheat hay from the three planting intervals. The distribution of the radioactive residues in wheat hay is summarised in Tables 34 and 35 for the PH-T and TH-T samples, respectively.

Table 34 Summary of Characterisation and Identification of Radioactive Residues in Wheat Hay Following Seed-Treatment Application of [Phenyl-^{14}C]Tioxazafen

	Wheat Hay – 30 day TRR 0.0315 mg eq/kg		Wheat Hay – 120 day TRR 0.0713 mg eq/kg		Wheat Hay – 360 day TRR 0.0228 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	0.5	0.0004	ND	NA
Benzoic acid	1.6	0.0005	2.9	0.0021	2.0	0.0005
Tioxazafen Imide	ND	NA	0.5	0.0004	ND	NA
Tioxazafen Iminoamide	3.5	0.0011	ND	NA	ND	NA
RT 18.3-18.8	14.0	0.0044	8.3	0.0059	5.2	0.0012
Benzamidine	2.8	0.0009	2.6	0.0019	5.4	0.0012
Maximum other single†	8.4	0.0026	13.1	0.0093	7.6	0.0017
Total identified	7.9	0.0025	6.5	0.0048	7.4	0.0017
Total characterised	57.4	0.0181	57.0	0.0406	34.4	0.0078
Total extracted	65.3	0.0206	63.5	0.0453	41.8	0.0095
Unextracted (PES)*	34.7	0.0109	36.5	0.0261	58.2	0.0133
Released by harsh treatments	32.4#	0.0102	24.2##	0.0173	45.8&	0.0105
Accountability**	120.9		100.1		124.5	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

Extraction with 0.1 M KOH (14.4% TRR, 0.0045 mg eq/kg) and 24% KOH (18.0% TRR, 0.0057 mg eq/kg).

Extraction with 0.1 M KOH (6.7% TRR, 0.0048 mg eq/kg) and 24% KOH (17.5% TRR, 0.0125 mg eq/kg).

& Extraction with 0.1 M KOH (6.1% TRR, 0.0014 mg eq/kg) and 24% KOH (39.7% TRR, 0.0091 mg eq/kg)

Table 35 Summary of Characterisation and Identification of Radioactive Residues in Wheat Hay Following Seed-Treatment Application of [Thiophene-^{14}C]Tioxazafen

	Wheat Hay – 30 day TRR 0.0552 mg eq/kg		Wheat Hay – 120 day TRR 0.0526 mg eq/kg		Wheat Hay – 360 day TRR 0.0339 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	ND	NA	ND	NA
2-Thiophenecarboxylic acid	6.5	0.0036	6.4	0.0034	4.6	0.0016
Tioxazafen Imide	0.9	0.0005	0.5	0.0003	ND	NA
RT 18.3-18.8	14.8	0.0082	12.1	0.0064	6.3	0.0021
Maximum other single†	7.4	0.0041	7.4	0.0039	9.6	0.0033
Total identified	7.4	0.0041	6.9	0.0036	4.6	0.0016
Total characterised	59.1	0.0326	59.0	0.0311	42.4	0.0144
Total extracted	66.5	0.0367	65.9	0.0347	47.0	0.0159
Unextracted (PES)*	33.5	0.0185	34.1	0.018	53.0	0.0179
Released by harsh treatments	29.9#	0.0165	26.5##	0.0140	40.5&	0.0137
Accountability**	98.5		96.2		112.3	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

Extraction with 0.1 M KOH (16.1% TRR, 0.0089 mg eq/kg) and 24% KOH (13.8% TRR, 0.0076 mg eq/kg).

Extraction with 0.1 M KOH (6.4% TRR, 0.0034 mg eq/kg) and 24% KOH (20.1% TRR, 0.0106 mg eq/kg).

& Extraction with 0.1 M KOH (6.8% TRR, 0.0023 mg eq/kg) and 24% KOH (33.7% TRR, 0.0114 mg eq/kg).

Residues in Wheat Straw:

The TRRs in wheat straw were highest at the 120-Day planting interval at 0.08-0.09 mg eq/kg and dropped off sharply to *ca* 0.02 mg eq/kg in both the PH-T and TH-T wheat straw at the 360-Day planting. The total extractable from the three PH samples ranged from 53-68% TRR, but HPLC analysis showed that there were a number of low level metabolites and none of the metabolites in the PH straw was prominent, except for the unknown metabolite at RT 18.3 which represented 6.3% TRR (0.0027 mg eq/kg) in the 30-Day straw. An unknown metabolite represented *ca* 12 and 11% TRR in the 120-day and 360-day PH-T wheat straw, but the mg eq/kg levels were ≤0.01 mg eq/kg. Trace levels (≤0.005 mg eq/kg) of Tioxazafen, Tioxazafen Imide, Tioxazafen Iminoamide, benzoic acid and benzamidine were quantified in wheat straw from the 30-Day and 120-Day plantings. A summary of the characterisation of the ^{14}C-residues in PH wheat straw is provided in Table 36.

Table 36 Summary of Characterisation and Identification of Radioactive Residues in Wheat Straw Following Seed-Treatment Application of [Phenyl-^{14}C]Tioxazafen

	Wheat Straw – 30 day TRR 0.0435 mg eq/kg		Wheat Straw – 120 day TRR 0.0769 mg eq/kg		Wheat Straw – 360 day TRR 0.0176 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	0.2	0.0001	0.5	0.0004	ND	NA
Benzoic acid	ND	NA	2.3	0.0018	ND	NA
Tioxazafen Imide	0.9	0.0004	1.6	0.0012	2.7	0.0005
Tioxazafen Iminoamide	ND	NA	3.4	0.0026	9.8	0.0017
RT 18.3-18.8	6.3	0.0027	6.4	0.0049	ND	NA
Benzamidine	2.3	0.0010	1.9	0.0015	ND	NA
Maximum other single†	6.6	0.0029	12.4	0.0095	11.0	0.0019
Total identified	3.4	0.0015	9.7	0.0075	12.5	0.0022
Total characterised	55.3	0.0240	43.5	0.0333	55.7	0.0098
Total extracted	58.7	0.0255	53.2	0.0408	68.2	0.0120
Unextracted (PES)*	41.3	0.0180	46.9	0.0360	31.8	0.0056
Released by harsh treatments	34.7#	0.0151	42.1##	0.0323	NA	NA
Accountability**	117.5		107.2		127.2	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

Extraction with 0.1 M KOH (10.2% TRR, 0.0044 mg eq/kg). Extraction with 24% KOH (24.5% TRR, 0.0107 mg eq/kg).

Extraction with 0.1 M KOH (14.6% TRR, 0.0112 mg eq/kg). Extraction with 24% KOH (27.5% TRR, 0.0211 mg eq/kg).

The TRR in TH wheat straw reached a maximum in the 120-day planting sample where it was 0.0869 mg eq/kg. Samples from all three planting intervals were extracted, with extractable radioactivity ranging from 51-58% TRR. The HPLC analyses of the extracts showed numerous poorly resolved peaks. In the analysis of the 30-day and 120-day wheat straw extracts, 2-thiophenecarboxylic acid was found at 3-5% TRR, while trace amounts of Tioxazafen (0.0004-0.0005 mg eq/kg (0.5-0.8% TRR)) were found. The unknown at RT 18.3-18.8 represented 7-10% TRR.

Table 37 Summary of Characterisation and Identification of Radioactive Residues in Wheat Straw Following Seed-Treatment Application of [Thiophene-^{14}C]Tioxazafen

	Wheat Straw – 30 day TRR 0.0592 mg eq/kg		Wheat Straw – 120 day TRR 0.0869 mg eq/kg		Wheat Straw – 360 day TRR 0.0220 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	0.8	0.0005	0.5	0.0004	ND	NA
2-Thiophenecarboxylic acid	5.3	0.0031	2.6	0.0023	ND	NA
Tioxazafen Imide	0.7	0.0004	ND	NA	ND	NA
2-Thiophenecarboxamide	ND	NA	1.6	0.0014	ND	NA
RT 18.3-18.8	6.7	0.0040	9.7	0.0084	ND	NA
Maximum other single†	6.7	0.0040	8.8	0.0076	7.9	0.0017
Total identified	6.8	0.0040	4.7	0.0041	0.0	NA
Total characterised	43.9	0.0260	49.8	0.0433	58.4	0.0128
Total extracted	50.7	0.0300	54.5	0.0474	58.4	0.0128
Unextracted (PES)*	49.2	0.0292	45.5	0.0396	41.6	0.0092
Released by harsh treatments	39.1#	0.0232	40.2##	0.0350	NA	NA
Accountability**	101.7		109.0		98.7	

ND, not detected; NA, not applicable * Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

†Represents the larges HPLC peak not otherwise listed

Extraction with 0.1 M KOH (12.1% TRR, 0.0072 mg eq/kg). Extraction with 24% KOH (27.0% TRR, 0.0160 mg eq/kg).

Extraction with 0.1 M KOH (14.8% TRR, 0.0129 mg eq/kg). Extraction with 24% KOH (25.4% TRR, 0.0221 mg eq/kg).

Residues in Wheat Grain:

The residues in wheat grain were very low. In both the PH and TH labels, the maximum residue occurred in the grain from the 120-day planting, in which it was 0.007 (PH) and 0.005 mg eq/kg (TH). HPLC analysis of the 30-day wheat grain showed the presence of traces of benzamidine (2.6% TRR, 0.0001 mg eq/kg) in the PH grain. No Tioxazafen was detected in either the PH or TH grain. The only unidentified metabolite in the 30-day wheat grain ≥10% TRR was present in extremely low concentrations (0.0006-0.0012 mg eq/kg) in both the PH and TH labels. The low level of extractable radioactivity combined with sample concentration difficulty prevented HPLC analysis of the 120-day TH-T wheat grain extract.

Table 38 Summary of Characterisation and Identification of Radioactive Residues in Wheat Grain Following Seed-Treatment Application of [Phenyl-^{14}C]Tioxazafen

	Wheat Grain – 30 day TRR 0.0039 mg eq/kg		Wheat Grain – 120 day TRR 0.0070 mg eq/kg		Wheat Grain – 360 day TRR 0.0029 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	NA	NA	NA	NA
Benzamidine	2.6	0.0001	NA	NA	NA	NA
Maximum other single†	30.8	0.0012	NA	NA	NA	NA
Total identified	2.6	0.0001	NA	NA	NA	NA
Total characterised	34.4	0.0013	NA	NA	NA	NA
Total extracted	37.0	0.0014	46.3	0.0032	NA	NA
Unextracted (PES)*	63.0	0.0025	53.7	0.0038	NA	NA
Released by harsh treatments	9.4#	0.0004	NA	NA	NA	NA
Accountability**	96.9		65.2		NA	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

†Represents the largest HPLC peak not otherwise listed # 0.1 M KOH

Table 39 Summary of Characterisation and Identification of Radioactive Residues in Wheat Grain Following Seed-Treatment Application of [Thiophene-^{14}C]Tioxazafen

	Wheat Grain – 30 day TRR 0.0035 mg eq/kg		Wheat Grain – 120 day TRR 0.0050 mg eq/kg		Wheat Grain – 360 day TRR 0.0032 mg eq/kg	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Tioxazafen	ND	NA	NA	NA	NA	NA
Maximum other single†	15.9	0.0006	NA	NA	NA	NA
Total characterised	32.5	0.0011	NA	NA	NA	NA
Total extracted	32.5	0.0011	NA	NA	NA	NA
Unextracted (PES)*	67.5	0.0023	NA	NA	NA	NA
Released by harsh treatments	12.4#	0.0004	NA	NA	NA	NA
Accountability**	100.9		NA		NA	

ND, not detected; NA, not applicable

* Residues remaining after neutral extractions.

** Accountability = (Total extractable + Total unextractable) / TRR from combustion analysis * 100

† Represents the largest HPLC peak not otherwise listed

0.1 M KOH

In summary, the TRR in lettuce, radish and wheat raw agricultural commodities, grown as rotational crops representing the leafy vegetable, root and grain crops, were very low after treatment of the confined plots by planting of maize seeds treated with radiolabelled tioxazafen as a seed treatment. In general, the highest residue for a given raw agricultural commodity was observed in the sample from the 120-day planting, and the residues then fell significantly in the 360-day planting. The highest TRRs were observed in the wheat straw from the 120-day planting in which the TRRs were 0.077 and 0.087 mg eq/kg in the PH and TH samples, respectively. Wheat grain TRRs were below 0.01 mg eq/kg at all plantings in both labels.

The levels of parent tioxazafen were very low in those cases in which it was detected. Very low levels of benzamidine, benzamide, benzoic acid, and tioxazafen tminoamide were found in several crop samples from the PH label. In the TH-labelled commodities, low levels of tioxazafen imide and iminoamide and 2-thiophenecarboxylic acid were detected.

A proposed metabolic pathway for MON 102100 in lettuce, radish and wheat based on metabolites identified or characterised, and by analogy to metabolism in other crops, is presented in Figure 6.

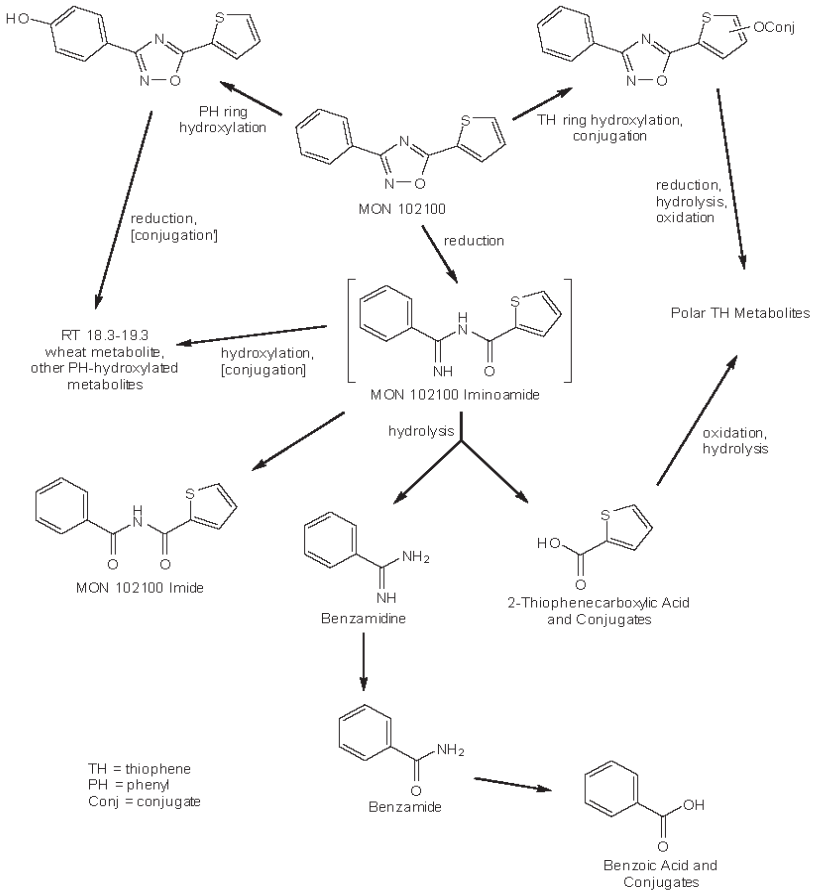


Figure 6 Proposed Metabolic Pathway for Tioxazafen in Confined Rotational Crops

Field Rotational Crop Studies

The meeting received a field rotational crop study (Urbanczyk-Wochniak and Riter 2015 MSL0026525).

Lettuce and radish were planted at 30-, 120- and 365-day plant-back intervals, sorghum was planted at the 30- and 365-day plant-back intervals, wheat was planted at the 120-day plant-back interval, in plots previously planted with tioxazafen treated soya bean seed (Asgrow variety AG6132) at rate of 0.568 mg ai/seed (equivalent to 0.29-0.35 kg ai/ha. Lettuce, radish, sorghum and wheat commodities were collected at the normal harvest time. The parent tioxazafen and main metabolites were by GC-MS/MS or LC-MS/MS. Average method recoveries range from 82.9 to 110% for Tioxazafen across all RACs, and from 82.7 to 116% for benzamidine.

The magnitude of tioxazafen and benzamidine residues in rotational crop RACs are summarised below in Table 40 for the 30-day plant-back interval.

Table 40 Summary of Residue Data from Field Trials on 30-Day Plant-back Interval Rotation Crops

Commodity	Site	PBI (Days)	DAP (Days)	Site-Averaged Residues (mg eq/kg)		
				Benzamidine	Tioxazafen	Total
Lettuce Leaves	01NC	29	52	<0.0025	0.0043	0.0068
	02CA	30	52	<0.0025	<0.0025	<0.0050
Radish Tops	01NC	29	39	0.0028	<0.0025	0.0053
	02CA	30	35	0.0038	<0.0025	0.0063
Radish Root	01NC	29	39	<0.0025	<0.0025	<0.0050
	02CA	30	35	<0.0025	<0.0025	<0.0050
Sorghum Forage	01NC	29	81	<0.0025	<0.0025	<0.0050
	02CA	30	118	<0.0025	<0.0025	<0.0050

Commodity	Site	PBI (Days)	DAP (Days)	Site-Averaged Residues (mg eq/kg)		
				Benzamidine	Tioxazafen	Total
Sorghum Grain	01NC	29	103	<0.0025	<0.0025	<0.0050
	02CA	30	151	<0.0025	<0.0025	<0.0050
Sorghum Stover	01NC	29	107	<0.0025	<0.0025	<0.0050
	02CA	30	151	<0.0025	<0.0025	<0.0050

Benzamidine residues are expressed as Tioxazafen equivalents. The LOQ of Tioxazafen and benzamidine is 0.0025 mg/kg (Tioxazafen equivalents).

Values below 0.0025 are shown as <0.0025. Values are based on averages of two replicates per trial.

PBI = Plant-Back Interval (interval from planting of primary crop to planting of rotational crop)

DAP = Days after Planting (interval from planting of rotational crop to harvest of commodity

Low levels of tioxazafen and benzamidine were only detected in the 30 day PBI lettuce leaf and radish top samples with no tixoazafen or benzimidine detected in any samples of radish root, sorghum and wheat forage, stover or grain. Samples from the 365 day PBI were not analysed as no residues were observed at the 120 day PBI.

ANIMAL METABOLISM

Lactating goat

A study on metabolism of Tioxazafen in lactating goats were available for the meeting (Quistad and LaMar 2014 MSL0024994).

Two lactating goats were dosed daily via gelatine capsules for five days with either the PH- or TH-labelled tioxazafen at a rate equivalent to 10.6 ppm in the feed. Milk, urine and feces were collected twice daily, in the morning before dosing and in the evening. The goats were sacrificed approximately 18-19 hr after administration of the last dose and liver, kidney, flank and loin muscle, omental subcutaneous and renal fat, GI tract with contents were removed for analysis. TRR was determined by direct combustion and LSC for feces and GI tract, by direct LSC analysis for urine and skim milk, and by solubilization (Soluene®) and LSC analysis for tissues and milk fat.

Liver, kidney, muscle (flank and loin) and feces were each extracted twice with 100 ml acetonitrile/water (1:1, v/v; 2×) followed by extraction once with 100ml acetonitrile (1×) to solubilize radioactive residues. The liver and kidney PES was extracted further with 0.1 M KOH and 4 M KOH, in sequence, or receied treatment with a nonspecific protease(37 °C for 48 hr). Milk was separated into skim milk and milk fat by centrifugation, and skim milk was extracted with 20ml acetone (1×) and acetone/water (1:1, v/v; 2×) followed by a final acetone extraction. Milk fat, omental fat, renal fat and subcutaneous fat were each extracted with 20ml hexane/acetone (4:1, 1×) followed by acetone (2×); the hexane/acetone extracts were concentrated and partitioned between hexane and ACN (3×). All TH-label samples of fat and muscle were not subjected to extraction as the TRR in each was < 0.001 mg eq/kg.

The total recovery of radiolabel was 90.9% (PH label) and 86.7% (TH label). Most of the administered dose was recovered in feces (64.5% for PH and 33.4% for TH) and urine (19.9% for PH and 49.6% for TH). The gastrointestinal tract with contents contained 5.41% and 2.86% of the administered dose for the PH and TH label, respectively. The highest tissue residue was found in the liver (0.334-1.095 mg eq/kg, 0.29-0.53% of the administered dose), followed by kidney (0.217-0.383 mg eq/kg, 0.03-0.04% of the administered dose). The other sample with significant residues was the milk fat (maximum 0.256-0.268 mg eq/kg). The TRR was lower for skim milk (maximum 0.032-0.083 mg eq/kg). Only 0.08-0.24% of the dose was excreted in milk, and milk residues appeared to plateau after the second dose. The TRR in muscle for the PH label was 0.052-0.055 mg eq/kg, but was <0.001 mg eq/kg for both the muscle and fat from the TH-label. The residues in fat from the PH label were low at 0.014-0.018 mg eq/kg for the renal, omental and subcutaneous fat.

The TRR values for the liver, kidney, muscle, fat and milk samples are given in Table 41.

Table 41 Distribution of the Radioactive Residue in the Tissues, Milk and Excreta from Goats Administered Tioxazafen

Matrix	[^{14}C]Tioxazafen			
	PH Label		TH Label	
	Percent of Total Dose	TRR (mg/kg equivalent)[a]	Percent of Total Dose	TRR (mg/kg equivalent)[a]
Tissues				
Liver	0.53	1.095	0.29	0.334
Kidney	0.04	0.383	0.03	0.217
Skim milk	0.05	0.026[b]	0.18	0.083[b]
Milk fat	0.03	0.256[b]	0.06	0.268[b]
Omental fat	0.01[c]	0.015	NA	<0.001
Subcutaneous fat	<0.01	0.018	NA	<0.001

Matrix	[^{14}C]Tioxazafen			
	PH Label		TH Label	
	Percent of Total Dose	TRR (mg/kg equivalent)[a]	Percent of Total Dose	TRR (mg/kg equivalent)[a]
Renal fat	<0.01	0.014	NA	<0.001
Flank muscle	0.01[c]	0.052	NA	<0.001
Loin muscle	0.03	0.055	NA	<0.001
Excreta				
Feces	64.50	-	33.43	-
G.I. tract and contents	5.41	-	2.86	-
Urine	19.93	-	49.55	-
Cage wash	0.35	-	0.25	-
Total Recovery	90.89	-	86.65	-

[a] mg/kg values taken from combustion data are parent equivalents.

[b] Based on Day 2 PM specimen.

[c] The percent of total dose for muscle and fat represents only the sample removed at Genesis Midwest for analysis.

NA = Not applicable

Only 0.08% (PH label) and 0.24% (TH label) of the administered dose was found in milk, the majority in skim milk. The distribution of the radioactivity in milk as a function of time is presented in Figure 7.

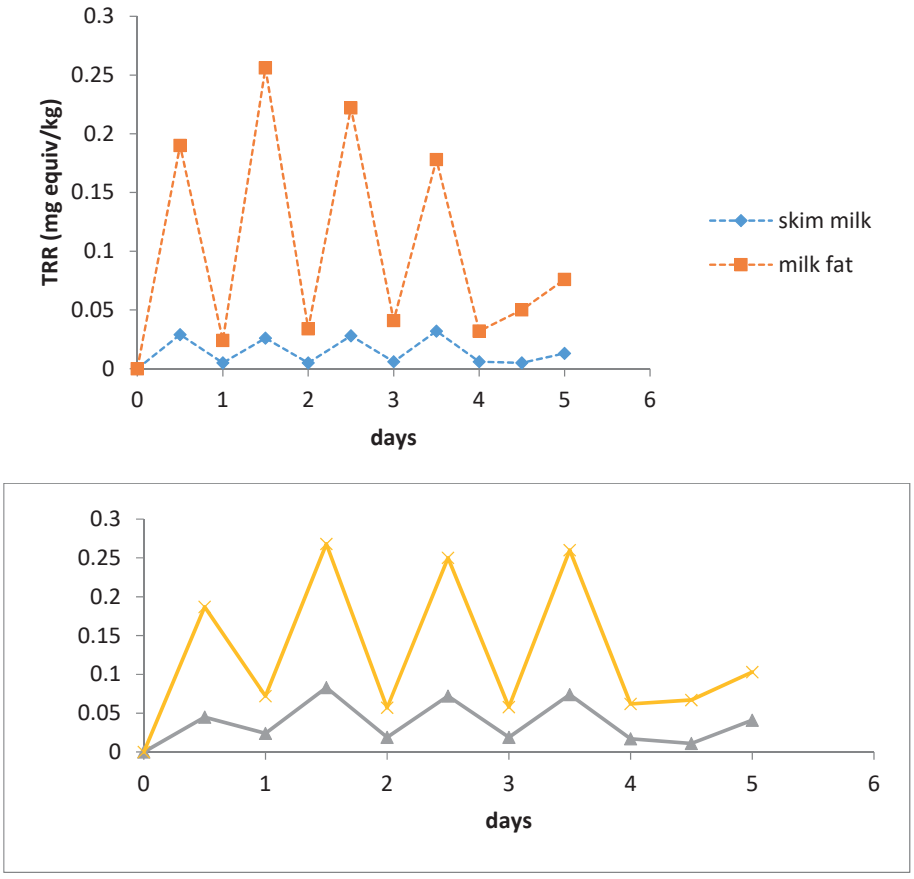


Figure 7 Residues in skim milk and milk fat following dosing with ^{14}C tioxazafen, a) PH label(up), b) TH-label(below)

A summary of the characterisation and identification of radioactive residues in goat liver, kidney, flank and loin muscle, and skim milk from the PH-treated goat is provided in Table 42. The summary of the characterisation and identification of radioactive residues in goat liver, kidney, skim milk and milk fat from the TH-treated goat is provided in Table 43.

Table 42 Summary of Characterisation and Identification of Radioactive Residues in Goat Liver, Kidney, Muscle and Skim Milk Following Administration of Phenyl-^{14}C-Tioxazafen at 10 ppm in Feed

	Liver TRR = 1.095 mg eq/kg		Kidney TRR = 0.383 mg eq/kg		Flank Muscle TRR = 0.052 mg eq/kg		Loin Muscle TRR = 0.055 mg eq/kg		Skim Milk TRR = 0.026 mg eq/kg	
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
ACN/water extracts	30.0	0.329	59.1	0.226	99.4	0.052	99.5	0.055	92.4	0.024
Tioxazafen	nd	na	nd	na	nd	na	nd	na	nd	na
Benzamidine	24.6	0.269	44.3	0.170	99.4	0.052	98.9	0.054	28.6	0.007
Benzamide	0.6	0.007	4.9	0.019	nd	na	nd	na	7.5	0.002
Benzoic acid	0.5	0.005	nd	na	nd	na	nd	na	2.2	0.001
Glucuronide conjugate	nd	na	nd	na	nd	na	nd	na	18.7	0.005
PES	70	0.767	41	0.157	0.6	<0.001	0.5	<0.001	7.6	0.002
KOH treatment of PES - Total	69.5	0.762	40.5	0.155	na	na	na	na	na	na
EtOAc Phase	17.3	0.189	11.4	0.044	na	na	na	na	na	na
Benzamidine	nd	na	nd	na	na	na	na	na	na	na
Benzamide	3.2	0.035	3.0	0.011	na	na	na	na	na	na
Benzoic Acid	12.0	0.131	7.0	0.027	na	na	na	na	na	na
Aqueous Phase	10.2	0.111	20.6	0.078	na	na	na	na	na	na
Benzamidine	5.3	0.058	8.7	0.033	na	na	na	na	na	na
Benzamide	1.5	0.016	2.6	0.010	na	na	na	na	na	na
Benzoic acid	nd	na	nd	na	na	na	na	na	na	na
Remaining Solids	42.0	0.460	8.5	0.033	na	na	na	na	na	na
Last Remaining [a]	0.5	0.005	0.5	0.002	0.6	<0.001	0.5	<0.001	7.6	0.002

[a] Residues remaining after exhaustive extractions nd = not detected, na = not applicable

Table 43 Summary of Characterisation and Identification of Radioactive Residues in Goat Liver, Kidney and Milk Following Administration of Thiophene-^{14}C- Tioxazafen at 10 ppm in Feed

	Liver TRR = 0.334mg eq/kg		Kidney TRR = 0.217mg eq/kg		Skim Milk TRR = 0.083mg eq/kg		Milk Fat TRR = 0.268mg eq/kg	
Compound	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg
ACN/water extracts	16.4	0.055	35.6	0.077	93.5	0.078	nd	na
Tioxazafen	nd	na	nd	na	nd	na	nd	na
2-Thiophenecarboxylic acid	1.1	0.004	1.5	0.003	nd	na	nd	na
Thenoylglycine	1.5	0.005	21.5	0.047	65.2	0.054	nd	na
Glucuronide conjugate	nd	na	nd	na	3.4	0.003	nd	na
Sulfate conjugate	nd	na	nd	na	nd	na	nd	na
Hexane/acetone extracts	nd	na	nd	na	nd	na	89.0[b]	0.238
Acetonitrile phase	nd	na	nd	na	nd	na	72.8	0.195
Tioxazafen	nd	na	nd	na	nd	na	nd	na
2-Thiophenecarboxylic acid	nd	na	nd	na	nd	na	nd	na
Thenoylglycine	nd	na	nd	na	nd	na	12.7	0.034
Glucuronide conjugate	nd	na	nd	na	nd	na	1.3	0.003
Sulfate conjugate	nd	na	nd	na	nd	na	55.8[c]	0.150
Hexane phase (triglycerides)	nd	na	nd	na	nd	na	16.2	0.043
PES	83.6	0.279	64.4	0.14	6.5	0.005	11.0	0.029
KOH Extraction of PES – Total	83.2	0.278	63.7	0.138	nd	na	11.0	0.029
Combined EtOAc Phase	6.0	0.020	4.6	0.010	nd	na	nd	na
Combined Aqueous Phase	15.1	0.050	50.0	0.109	nd	na	nd	na
Combined Solids Phase	62.1	0.207	9.1	0.020	nd	na	nd	na
Remaining [a]	0.4	0.001	0.7	0.002	6.5	0.005	nd	na

[a] Residues remaining after exhaustive extractions

[b] Represents the sum of the acetonitrile phase and the hexane phase

[c] In whole milk, the sulfate conjugate represented 13.9% TRR (0.014 mg/kg)

nd = not detected, na = not applicable

Characterisation of Radioactive Residue in Liver

PH Liver:

The TRR in PH liver was 1.095 mg/kg, of which 30% was contained in the ACN/water extractions. Tioxazafen was not detected in the HPLC analysis. The major extracted residue in PH liver was benzamidine (24.6% TRR, 0.269 mg/kg), confirmed by TLC. Low amounts of benzoic acid (0.5% TRR, 0.005 mg/kg) and benzamide (0.6% TRR, 0.007 mg/kg) were also detected.

The initial PES was further treateded with 0.1 M KOH (23.7% released), and then with 4 M KOH (45.8% released). Each of these basic extracts was individually acidified (pH 2) and partitioned with ethyl acetate. Acidification resulted in precipitation of 11.8% TRR for the 0.1 M KOH sample and 30.2% TRR for the 4 M KOH sample. Equal portions of the ethyl acetate phases from the KOH treatments were combined (17.3% TRR) and analysed by HPLC to detect benzamide (0.035 mg/kg, 3.2% TRR) and benzoic acid (0.131 mg/kg, 12.0% TRR), which were confirmed by TLC. Equal portions of the aqueous phases were combined (10.2% TRR), and analysed by HPLC with detection of benzamide (0.016 mg/kg, 1.5% TRR) and benzamidine (0.058 mg/kg, 5.3% TRR).

Another sample of PH liver was extracted with ACN/water to provide PES (0.830 mg/kg, 75.8% TRR) for treatment with Pronase, a non-specific protease, to solubilize protein into constituent peptides and amino acids. This treatment released 0.245 mg eq/kg (22.4% TRR) as solubilized radioactivity from proteins. A control sample incubated under the same conditions without Pronase released 0.071 mg/kg (6.5% TRR), suggesting that at least 21% of the residues in the liver PES is closely associated with protein (either occluded, covalently bound, or incorporated into small molecules from extensive metabolism of tioxazafen into constituent amino acids).

TH Liver:

The TRR was 0.334 mg/kg, of which 0.055 mg/kg (16.4% TRR) was extracted with acetonitrile/water. Tioxazafen was not detected in the HPLC analysis, the profile of which was considerably more complex than the PH liver profile, and exhibited a multitude of low-level components. Only low amounts of 2-thiophenecarboxylic acid (0.004 mg/kg, 1.1% TRR) and its glycine conjugate, thenoylglycine (0.005 mg/kg, 1.5% TRR), were detected.

Further treatment of the PES with 0.1 M KOH and then with 4 M KOH released 39.2% TRR and 44.0% TRR, respectively. Acidification of the extracts to pH 2 resulted in precipitation of 26.9% TRR and 35.2% TRR, for the 0.1 M and 4 M KOH extracts, respectively. Partitioning with ethyl acetate resulted in 15.1% TRR in the combined aqueous phases and only 6% TRR in the combined organic phases.

Another sample of TH liver was extracted to provide PES for treatment with Pronase. Treatment of the TH liver PES (0.279 mg/kg, 83.6% TRR) released 0.082 mg/kg (24.7% TRR) as solubilized radiolabel from proteins. A control sample gave 0.013 mg/kg (4.0% TRR) when incubated under the same conditions. Most of the radiolabel (0.184 mg/kg, 55.1% TRR) was not solubilized by Pronase. The results of the Pronase digestion suggest that at least 25% of the TH liver PES is closely associated with protein.

Characterisation of Radioactive Residues in Kidney

PH Kidney:

The TRR in PH kidney was 0.383 mg/kg, of which 0.226 mg/kg (59.1% TRR) was extracted with ACN/water. Tioxazafen was not detected in the HPLC analysis. The major residue was benzamidine (0.170 mg/kg, 44.3% TRR), which was confirmed by TLC. A small amount of benzamide (0.019 mg/kg, 4.9% TRR) was also detected.

Further treatment of the PES with 0.1 M KOH and 4 M KOH released 23.3% TRR and 17.2% TRR, respectively. Acidification to pH 2 of the extracts resulted in precipitation of 8.5% TRR for the 4 M KOH sample. After partitioning of the acidified extracts with ethyl acetate, equal portions of the organic phase were combined and analysed by HPLC to give benzamide (0.011 mg/kg, 3.0% TRR) and benzoic acid (0.027 mg/kg, 7.0% TRR), which were confirmed by TLC. Equal portions of the aqueous phases were combined and analysed by HPLC with benzamide (0.010 mg/kg, 2.6% TRR) and benzamidine (0.033 mg/kg, 8.7% TRR) detected.

Another sample of PH kidney was extracted with acetonitrile/water to provide PES for Pronase treatment. Treatment of this PES (0.158 mg/kg, 41.2% TRR) released 0.098 mg/kg (25.7% TRR) as solubilized radioactivity from proteins. A control sample gave 0.025 mg/kg (6.5% TRR) under the same incubation conditions. The results of the Pronase digestion suggest that

at least 46% of the PES is closely associated with protein. Some of the radioactivity (0.035 mg/kg, 9.1% TRR) was not solubilized by Pronase.

TH Kidney:

The TRR of TH kidney was 0.217 mg/kg, of which 0.077 mg/kg (35.6% TRR) was extracted with ACN/water. Analysis by HPLC of the extract showed that thenoylglycine (0.047 mg/kg, 21.5% TRR) was the major extractable component. No tioxazafen was detected, but a small amount of unconjugated 2-thiophenecarboxylic acid (0.003 mg/kg, 1.5% TRR) was found. Thenoylglycine was confirmed by TLC.

The initial PES was further sequentially treated with 0.1 M KOH and 4 M KOH, releasing a further 46.1% and 17.6% TRR, respectively. After acidification of each of the basic extracts and partitioning with ethyl acetate, the combined aqueous phases contained 50% TRR, and the combined ethyl acetate phases contained only 4.6% TRR. Acidification of the 4 M KOH extract resulted in precipitation of 9.1% TRR.

Another sample of TH kidney was extracted with acetonitrile/water to provide PES for Pronase treatment. Treatment of this PES (0.146 mg/kg, 67.4% TRR) released 0.077 mg/kg (35.5% TRR) as solubilized radioactivity from proteins. A control sample gave 0.007 mg/kg (3.0% TRR) under the same incubation conditions. The results of the Pronase digestion suggest that at least 48% of the PES is closely associated with protein. Much of the radioactivity (0.062 mg/kg, 28.6% TRR) was not solubilized by Pronase.

Characterisation of the Radioactive Residue in Muscle

PH Label:

Flank muscle was extracted with acetonitrile/water (2X) and acetonitrile (1X); the combined extracts contained 0.052 mg/kg (99.4% TRR). Tioxazafen was not detected in the HPLC analysis, and the single major residue was identified as benzamidine (99.4% TRR) based on its retention time. TLC showed that the metabolite was benzamidine, not benzamide oxime which has a similar retention time. Positive confirmation of benzamidine was obtained by a second TLC method.

Loin muscle was extracted as described for flank muscle. The combined extracts contained 99.5% TRR. Analysis by HPLC showed that no Tioxazafen was detected, and that the major residue observed coincided to the retention time of benzamidine. TLC also showed that the residue was benzamidine, not benzamide oxime, and a second TLC method confirmed the residue was benzamidine.

TH Label:

No extraction and analysis of TH flank and loin muscle was conducted because the TRR was < 0.001 mg/kg in each of these matrices.

Characterisation of Radioactive Residues in Milk

Whole milk was separated into milk fat and skim milk for analysis, and the residues in whole milk were determined by summing the residues in skim milk and milk fat. Since the mass of skim milk is much greater than the mass of milk fat, the overall residues in whole milk are similar to that in skim milk. Although most of the radioactivity was in skim milk, the concentration was higher in milk fat. Representative milk samples with high residue (Day 2 PM) were analysed as described below.

PH Skim Milk:

Acetone/water extracts of skim milk contained 0.024 mg eq/kg (92.4% TRR **Table** 4 2). There were two major residues in the HPLC analysis: benzamidine (0.007 mg/kg, 28.6% TRR, confirmed by 2D-TLC) and a metabolite eluting at 34 minutes, RT34 (0.005 mg/kg, 18.7% TRR). On a whole milk basis, the benzamidine was present at a level of 0.007 mg/kg (16.3% TRR). The unknown at RT34 was isolated for further analysis. Incubation of the RT34 isolate in phosphate buffer gave no change, while addition of ß-glucuronidase resulted in formation of a less polar peak at RT 40 min, suggesting that RT34 was a glucuronic acid conjugate. The retention time of RT34 is consistent with that of a major rat metabolite of tioxazafen that has been identified as a glucuronide of hydroxy tioxazafen in which hydroxylation has occurred at the 5-position of the thiophene ring. Attempts to confirm this proposed structure by methylation with trimethylsilyldiazomethane were unsuccessful as the metabolite appeared to decompose during the procedure.

PH Milk Fat:

Milk fat (TRR = 0.256 mg eq/kg) was extracted with acetone/hexane, which was then concentrated and partitioned with acetonitrile and hexane. The partitioning resulted in 77.4% TRR (0.198 mg/kg) in the acetonitrile phase. The hexane phase contained 10.4% TRR (0.027 mg/kg). Analysis of the acetonitrile extract by HPLC showed that no tioxazafen was detected,

and that there was a single major metabolite (RT 40 min, 0.174 mg/kg, 67.8% TRR), whose retention time did not correspond to any of the available reference standards. The level of this metabolite in milk fat corresponded to a level of 0.013 mg/kg (30.2% TRR) in whole milk. The maximum of any other single metabolite was 0.002 mg/kg (0.8% TRR). Although that metabolite had a retention time similar to the tioxazafen imide reference standard, TLC showed little radioactivity in that region. The metabolite at *ca* 33.8 min was proposed to be the glucuronide conjugate of tioxazafen (RT34) based on comparison to skim milk.

The major unknown (RT40) was purified by HPLC for LC-MS analysis, which gave a major peak at RT 34 min. The negative ion ESI mass spectrum of this peak showed doublet ions at *m/z* 323 and 324 $[M-H]^-$, with the doublet corresponding to the 1:1 proportion of the ^{12}C and ^{13}C-tioxazafen in the test substance. The selected ion chromatograms corresponding to these ions confirmed that the 34-minute retention time analyte co-eluted with the radioactivity. MS/MS analysis of the *m/z* 323 ion gave *m/z* 243 (loss of 80 amu). The positive ion ESI mass spectrum displayed doublet ions at *m/z* 325 and 326, corresponding to the parent ion, and fragment ions at *m/z* 245 and 246 corresponding to the loss of 80 amu. Selected ion monitoring for the *m/z* 245 ion showed a peak at 34 min indicating co-elution with the radioactivity. The loss of 80 amu in the mass spectral analyses is indicative of the loss of SO_3 from a sulfate conjugate. The RT 40 metabolite was also observed in the TH-label milk fat, indicating the presence of both labels in the metabolite, further supporting the proposed identification of a sulfate conjugate of hydroxy-tioxazafen for RT 40. The exact position of the hydroxyl group is not known but is likely the 5-position of the thiophene ring by analogy to known mammalian metabolism pathways of substituted thiophenes. Hydrolysis of the metabolite primarily to benzoic acid also supports hydroxylation on the thiophene ring. The level of the sulfate conjugate in milk fat corresponded to 0.013 mg/kg (30.2% TRR) in whole milk.

TH Skim Milk:

The TRR for skim milk was 0.083 mg/kg. The HPLC analysis of the extract showed a single major residue that was proposed to be thenoylglycine (0.054 mg/kg, 65.2% TRR) based on its retention time correspondence with the authentic reference standard. The level of this metabolite in skim milk corresponded to 0.052 mg/kg (51.5% TRR) in whole milk. TLC confirmed that the metabolite was thenoylglycine. By comparison to the HPLC of skim milk from the PH label, a glucuronide was also proposed based on its 34-minute retention time. 72.8

TH Milk Fat:

The TRR of TH milk fat was 0.268 mg/kg. Tioxazafen was not detected, but there were two major metabolites: thenoylglycine (0.034 mg/kg, 12.7% TRR) and the RT 40 min metabolite (0.150 mg/kg, 55.8% TRR) identified as a sulfate conjugate in PH milk fat. The level of this metabolite in milk fat corresponded to 0.014 mg/kg (13.9% TRR) in whole milk. The RT40 metabolite almost completely disappeared after sulfatase treatment while the thenoylglycine peak was unaffected; supporting the identification of the RT 40 metabolite as the sulfate conjugate of hydroxylated Tioxazafen. By comparison to the HPLC analysis of milk fat from the PH label, a glucuronide (RT34) was also found at very low levels (0.003 mg/kg, 1.3% TRR). The hexane phase contained 0.043 mg/kg (16.2% TRR).

Characterisation of the Radioactive Residue in Fat

PH Label:

A summary of the characterisation and identification of residues of phenyl-^{14}C-Tioxazafen in goat fat and milk fat is provided in **Table** 44. Omental fat was extracted with hexane/acetone and the concentrated extract was partitioned with acetonitrile and hexane. The acetonitrile phase (0.008 mg/kg, 50.2% TRR) was analysed by HPLC. Tioxazafen represented 0.001 mg/kg (9.4% TRR), and was confirmed by TLC. Benzamidine was the major residue (0.004 mg/kg, 25.6% TRR) and was confirmed by TLC. Benzonitrile represented 0.001 mg/kg (8.3% TRR) and was identified by co-injection of a reference standard; it could not be confirmed by TLC because of its volatility, and because of its very low levels, further spectral or chromatographic techniques were not attempted. Benzonitrile was, however, observed as a fat metabolite in laying hens, and its identity was confirmed by a second chromatographic technique, thereby lending support to this proposed structure. The polar radioactivity near the solvent front (0-15 min) was isolated by preparative HPLC to further examine this broad region of radioactive residue. Analysis of this isolated radioactivity by TLC and HPLC confirmed benzamidine as the only metabolite eluting in this region. The hexane phase contained 0.002 mg/kg (13.6% TRR). Due to the very low residue concentration, the nature of the radioactivity in the hexane phase was not examined, but may represent incorporation of ^{14}C into triglycerides by extensive metabolism of Tioxazafen.

Subcutaneous fat (TRR = 0.018 mg/kg) was extracted and partitioned as for the omental fat. The acetonitrile phase (0.011 mg/kg, 63.4% TRR) was analysed by HPLC, which showed that Tioxazafen represented 0.002 mg/kg (10.7% TRR), and that benzamidine was the major residue (0.007 mg/kg, 38.5% TRR); both of these were confirmed by TLC. Benzonitrile represented 0.001 mg/kg (3.9% TRR). The maximum of any unidentified metabolite was <0.001 mg/kg (2.0% TRR).

Renal fat (TRR = 0.014 mg/kg) was extracted and partitioned as for the omental fat. The acetonitrile phase (0.008 mg/kg, 57.8% TRR) was analysed by HPLC, which showed that benzamidine was the major residue (0.008 mg/kg, 55.9% TRR). A small amount of benzonitrile (<0.001 mg/kg, 1.9% TRR) was also observed. The hexane partitioning phase contained 0.001 mg/kg (7.4% TRR). Extraction of the PES with 0.1 M KOH yielded 0.004 mg/kg (26.6% TRR).

TH Label:

No extraction and analysis of TH-treated omental, subcutaneous, and renal fat tissues was conducted because the TRR was <0.001 mg/kg in each of these matrices.

Table 44 Summary of the Characterisation and Identification of Radioactive Residues in Goat Fat and Milk Fat Following Administration of Phenyl-^{14}C-Tioxazafen

Compound	Omental Fat TRR = 0.015 mg/kg		Subcutaneous Fat TRR = 0.018 mg/kg		Renal Fat TRR = 0.014 mg/kg		Milk Fat TRR = 0.256 mg/kg	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Hexane/acetone extracts	63.8[b]	0.010	74.0[b]	0.013	65.2[b]	0.009	87.8[b]	0.225
Acetonitrile phase	50.2	0.008	63.4	0.011	57.8	0.008	77.4	0.198
Tioxazafen	9.4	0.001	10.7	0.002	nd	na	nd	na
Benzamidine	25.6	0.004	38.5	0.007	55.9	0.008	nd	na
Benzonitrile	8.3	0.001	3.9	0.001	1.9	<0.001	nd	na
Sulfate conjugate	nd	na	nd	na	nd	na	67.8[c]	0.174
Glucuronide conjugate	nd	na	nd	na	nd	na	3.9	0.010
Hexane phase (triglycerides)	13.6	0.002	10.6	0.002	7.4	0.001	10.4	0.027
PES	36.2	0.006	26	0.003	34.8	0.005	12.1	0.031
0.1 M KOH	23.9	0.004	17.4	0.003	26.6	0.004	12.1	0.031
Remaining [a]	12.3	0.002	8.6	0.002	8.2	0.001	nd	na

[a)] Residues remaining after exhaustive extractions

[b)] Represents the sum of the acetonitrile phase and the hexane phase

[c)] In whole milk, the sulfate conjugate represented 30.2% TRR (0.013 mg/kg)

nd = not determined, na = not applicable

Characterisation of the Radioactive Residue in Urine and Faeces

In the excreta, 19.9-49.6% and 33.4-64.5% of the administered dose was excreted in the urine and faeces, respectively. A summary of the characterisation and identification of radioactive residues in goat urine and faeces following administration of phenyl- or thiophene-^{14}C-Tioxazafen is shown in Table 45.

Table 45 Summary of Residues from [^{14}C]Tioxazafen in Goat Urine and Faecal Extract Composites

Compound	Urine PH TRR = 12.47 mg/kg		Urine TH TRR = 11.95 mg/kg		Faeces PH TRR = 13.78 mg/kg		Faeces TH TRR = 5.64 mg/kg	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Tioxazafen	nd	na	nd	na	nd	na	nd	na
Benzamidine	11.57	92.8	na	na	6.90	50.1	na	na
Benzoic acid	0.262	2.1	na	na	0.386	2.8	na	na
Benzamide	0.274	2.2	na	na	0.220	1.6	na	na
Benzamidoxime	0.474	3.8	na	na	nd	na	na	na
Hippuric acid	0.374	3.0	na	na	nd	na	na	na
2-Thiophenecarboxylic acid	na	na	0.120	1.0	na	na	3.61	64.1
Thenoylglycine	na	na	11.17	93.5	na	na	nd	na
Unextractable (PES)	na	na	na	na	5.85	42.5	2.02	35.9

na = not applicable

nd = not detected

Analysis of PH Label Faeces:

The TRR for a composite sample of faeces was 13.78 mg/kg. HPLC analysis showed that no Tioxazafen was present in faeces, and that the most abundant metabolite was benzamidine (6.90 mg/kg, 50.1% TRR), with lesser amounts of benzoic

acid (0.386 mg/kg, 2.8% TRR) and benzamide (0.220 mg/kg, 1.6% TRR). These metabolites were confirmed by TLC. The PES contained 5.85 mg/kg (42.5% TRR).

Analysis of TH Label Faeces:

The TRR for a composite sample of faeces was 5.64 mg/kg. HPLC analysis showed that no Tioxazafen was present and that the major metabolite was 2-thiophenecarboxylic acid (3.61 mg/kg, 64.1% TRR), confirmed by TLC. The PES contained 2.02 mg/kg (35.9% TRR).

Analysis of PH Label Urine:

The TRR for a composite urine sample was 12.47 mg/kg. HPLC analysis revealed that most of the radioactive residue in urine corresponded to benzamidine (11.57 mg/kg, 92.8% TRR) with small amounts of benzoic acid (0.262 mg/kg, 2.1% TRR), benzamide (0.274 mg/kg, 2.2% TRR) and hippuric acid (0.374 mg/kg, 3.0% TRR), the glycine conjugate of benzoic acid and a known mammalian urinary metabolite of benzoic acid. No Tioxazafen was observed in the HPLC analysis of urine. These metabolites, except for hippuric acid, were confirmed by TLC, which also confirmed benzamide oxime as a minor component of the benzamidine peak observed by HPLC.

Analysis of TH Label Urine:

The TRR for the TH urine composite was 11.95 mg/kg. HPLC analysis gave mostly thenoylglycine, the glycine conjugate of 2-thiophenecarboxylic acid (11.17 mg/kg, 93.5% TRR), and a trace of unconjugated 2-thiophenecarboxylic acid (0.120 mg/kg, 1.0% TRR), both of which were confirmed by TLC.

In summary, benzamidine is a major residue in all tissues and is also hydrolyzed to benzamide and further to benzoic acid, which are minor metabolites. Benzoic acid is conjugated with glycine to hippuric acid. Other minor observed metabolites that are likely formed from benzamidine are benzonitrile (formed by elimination of ammonia) and benzamidoxime (formed by oxidation). The metabolite 2-thiophenecarboxylic acid is a minor residue in tissues but is the major metabolite observed in feces and is converted to its glycine conjugate, thenoylglycine, a major metabolite in urine and a significant metabolite in milk and kidney.

As a minor pathway, tioxazafen is hydroxylated (on the thiophene ring, but exact position unknown) followed by sulfation to a sulfate conjugate recovered in milk fat. This sulfate conjugate decomposes to hydroxy Tioxazafen when whole milk is extracted. A minor metabolite in milk (primarily in the skim milk fraction) was also characterised as a glucuronide, likely the glucuronide of the identical hydroxy Tioxazafen metabolite identified as the sulfate in milk fat.

A schematic of the proposed metabolic pathways is provided in Figure 8.

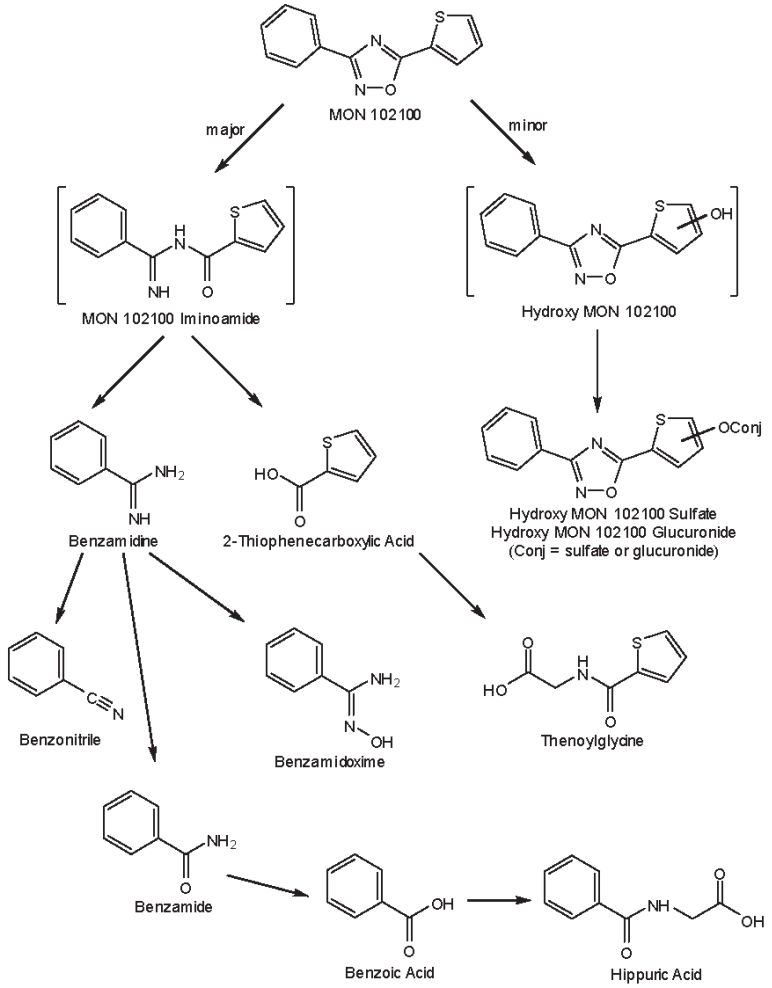


Figure 8 Proposed Pathways of Metabolism of Tioxazafen in Lactating Goats

Laying hens

The meeting received a study on metabolism of Tioxazafen in laying hen (LaMar and Quistad 2014 MSL0024995). The 37-week laying hens (*Gallus gallus domesticus*) were daily administered with either phenyl-^{14}C- or thiophene-^{14}C-tioxazafen via cellulose-filled gelatin capsule for seven days at a level of 10 ppm in the feed. Eggs and excreta were collected twice daily. Hens were sacrificed approximately 19-21.5 hr after the last dose was administered; the liver, breast and thigh muscle, abdominal and subcutaneous fat, and GI tract with contents were collected at sacrifice. TRR was determined by direct combustion and LSC for excreta and homogenized GI tracts. The TRR in liver, muscle, eggs and fat was measured by solubilisation of subsamples with Soluene™ tissue solubiliser. Radioactivity in cage washes was determined directly by LSC.

Liver, muscle, egg and excreta samples were each extracted with aqueous acetonitrile/water (1:1, v/v, 2×) and acetonitrile (1×) to solubilize radioactive residues. Fat was extracted with acetone/hexane (1:4, v/v, 1×) and then with acetone (2×). Separate sub-samples of post-extraction solids (PES) of liver and eggs were further extracted with 0.1 M KOH followed by 24% KOH, and with Pronase. Radioactive residues in tissue and excreta aqueous acetonitrile extracts were analysed by high performance liquid chromatography (HPLC). Egg acetonitrile-soluble fractions were analysed by HPLC, and the hexane phase from partitioning was analysed by thin layer chromatography (TLC). Metabolites were identified primarily by co-chromatography with reference standards, and their identities were confirmed by 1D- or 2D-TLC. Gas chromatography/mass spectrometry (GC/MS) was also utilised for metabolite identification.

The total recovery of the radioactivity was 90-91% of the administered dose, with 88-89% of the dose recovered in the excreta and 1-2% recovered in the gastrointestinal tracts at sacrifice. Cage washes accounted for 0.4-0.5% of the administered radioactivity. Recovery of radioactivity in tissues was generally low, with the highest residue in the liver at

0.61-0.66 mg/kg (0.32-0.33% of the administered dose), followed by fat (0.039-0.046 ppm, 0.01-0.02% of dose) and muscle (0.009-0.015 ppm, 0.01% of dose).Only 0.33-0.36% of the administered dose was excreted in eggs, with the peak residue levels in eggs found at the Day 7 and Day 8 samplings at 0.15-0.18 mg/kg; the increase of the residue level with time is consistent with the formation of the egg over a period of eight to nine days. Residues in fat ranged from 0.04-0.05 mg/kg for both labels. Residues in muscle were very low, ranging from 0.009 to 0.015 mg/kg.

17.1-19.7% TRR for liver, 33.5-48.3% TRR for muscle, and 87.6-91.8% TRR for fat were extrated. 21.6-27.3% TRR in egg samples from Day 5 AM and Day 8 AM were extracted.

The TRR values for the liver, muscle, fat and excreta samples are provided in Table 46.

Table 46 Distribution of the Radioactive Residue in the Tissues, Eggs and Excreta from Laying Hens Administered Radiolabelled Tioxazafen

Tissues	[^{14}C]Tioxazafen Residues			
	PH Label		TH Label	
	Percent of Total Dose	TRR (mg/kg eq)[a]	Percent of Total Dose	TRR (mg/kg eq)[a]
Liver	0.32	0.612	0.33	0.664
Thigh Muscle	0.01	0.014	0.01	0.015
Breast Muscle	0.01	0.009	0.01	0.009
Abdominal Fat	0.02	0.045	0.01	0.046
Subcutaneous Fat	0.01	0.044	0.01	0.039
Eggs[b]	0.33	-	0.36	-
Excreta	88.5	-	87.7	-
Gastrointestinal Tract and Contents	1.1	-	1.5	-
Cage wash	0.4	-	0.5	-
Total Recovery	90.7		90.4	

a) mg/kg values taken from solubilisation data are parent equivalents.

b) ^{14}C-residue in eggs from solubilisation ranged from 0.003-0.177 mg/kg

The distribution of the radioactive residues in eggs from each dosing group over the course of the study is provided in figure 9. Residue levels in eggs ranged from ND (not detected, Day 1 PM) to 0.149 mg/kg (Day 8 AM, PH label) and 0.177 mg/kg (Day 7 PM, TH label). Radiolabelled residue levels in eggs were relatively the same between groups for the same time point, but the residue gradually increased with time, a pattern which is consistent with the formation of an egg over a period of eight to nine days. Eggs accounted for 0.33% and 0.36% of the total administered radioactivity for the PH and TH radiolabels, respectively.

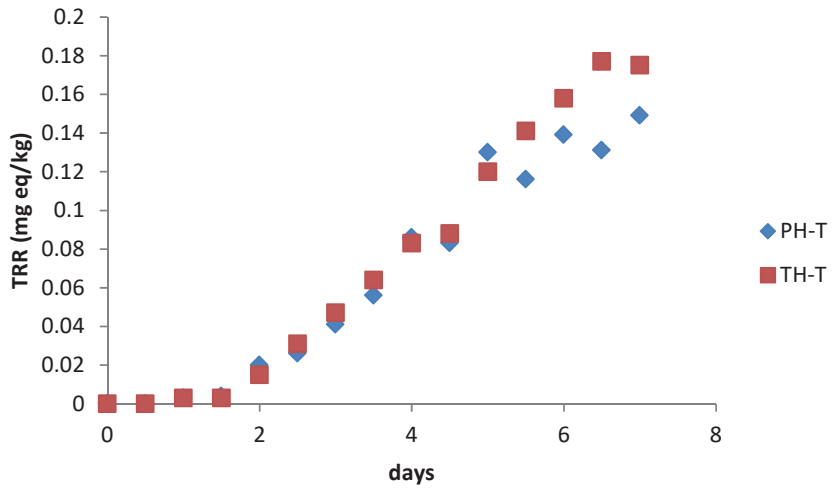


Figure 9 Residues in eggs following dosing with ^{14}C tioxazafen

The quantitation of the metabolites from those analyses is presented in Table 47 for the PH-dosed hens and Table 48 for the TH-dosed hens.

Table 47 Distribution of the TRR in Tissues from the PH-Dosed Hens

Compound	Liver TRR = 0.612 mg/kg			Breast Muscle TRR = 0.009 mg/kg		Thigh Muscle TRR = 0.014 mg/kg		Abdominal Fat TRR = 0.045 mg/kg		Subcutaneous Fat TRR = 0.044 mg/kg	
	%TRR	mg/kg		%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Combined initial extracts [a]	17.1	0.105		48.3	0.004	44.8	0.006	91.6	0.041	91.8	0.040
Aq ACN soluble	na	na		na	na	na	na	47.9	0.022	61.2	0.027
Tioxazafen	0.3	0.002		3.7	0.0003	5.6	0.001	12.0	0.005	18.7	0.008
Benzamidine	5.8	0.035		18.1	0.002	17.5	0.002	nd	na	nd	na
Benzamide	1.2	0.007		nd	na	nd	na	nd	na	nd	na
Tioxazafen Imide	1.7	0.010		nd	na	nd	na	nd	na	nd	na
Glucuronide conjugate (M1)	1.3	0.008		4.7	0.0004	2.7	0.0004	nd	na	nd	na
Benzonitrile	nd	na		nd	na	nd	na	21.2	0.010	30.1	0.013
Hexane soluble [b]	na	na		na	na	na	na	43.7	0.020	30.6	0.013
PES											
KOH treatment	83.0	0.508		na	na	na	na	na	na	na	na
EtOAc Phase	20.1	0.123		na	na	na	na	na	na	na	na
Benzoic acid	14.6	0.089		na	na	na	na	na	na	na	na
Aqueous phase	11.4	0.069		na	na	na	na	na	na	na	na
Benzoic acid	0.6	0.004		na	na	na	na	na	na	na	na
Benzamidine	2.0	0.012		na	na	na	na	na	na	na	na
Benzamide	1.3	0.008		na	na	na	na	na	na	na	na
Solids-total	51.5	0.315		na	na	na	na	na	na	na	na
Remaining [c]	0.0	0.0		51.7	0.005	55.2	0.008	8.4	0.004	8.2	0.004

[a] Amount extracted using initial extraction solvents [ACN/H_2O), 1:1 (2×) and ACN (1×) for liver, muscle and eggs; hexane/acetone, 4:1 (1×) and acetone (2×) for fat]

[b] Major components by TLC is Tioxazafen, does not separate from fat

[c] Residues remaining after exhaustive extractions of liver, or after normal extractions for other samples

na = not applicable

nd = not detected

Table 48 Distribution of the TRR in the Tissues from TH-Dosed Hens

Compound	Liver TRR = 0.664 mg/kg		Breast Muscle TRR = 0.009 mg/kg		Thigh Muscle TRR = 0.015 mg/kg		Abdominal Fat TRR = 0.046 mg/kg		Subcutaneous Fat TRR = 0.039 mg/kg	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Combined initial extracts [a]	19.7	0.131	34.3	0.003	33.5	0.005	87.6	0.040	90.6	0.035
ACN soluble	na	na	na	na	na	na	36.4	0.017	35.3	0.014
Tioxazafen	0.5	0.003	nd	na	3.7	0.001	20.7	0.010	20.4	0.008
Tioxazafen Imide	1.4	0.009	nd	na	nd	na	nd	na	nd	na
Glucuronide conjugate (M1)	0.8	0.005	2.6	0.0002	2.7	0.0004	nd	na	nd	na
M4 (thiophene specific)	3.5	0.023	13.2	0.001	11.8	0.002	nd	na	nd	na

Compound	Liver TRR = 0.664 mg/kg		Breast Muscle TRR = 0.009 mg/kg		Thigh Muscle TRR = 0.015 mg/kg		Abdominal Fat TRR = 0.046 mg/kg		Subcutaneous Fat TRR = 0.039 mg/kg	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Hexane soluble [b]	na	na	na	na	na	na	51.2	0.024	55.3	0.022
PES										
KOH treatment	80.4	0.534	na	na	na	na	na	na	na	na
EtOAc Phase	3.0	0.02	na	na	na	na	na	na	na	na
Aqueous phase	23.5	0.157	na	na	na	na	na	na	na	na
Solids	53.9	0.358	na	na	na	na	na	na	na	na
Remaining [c]	0.0	0.0	65.7	0.006	66.5	0.010	12.4	0.006	9.4	0.004

[a] Amount extracted using initial extraction solvents [ACN/H_2O), 1:1 (2×) and ACN (1×) for liver, muscle and eggs; hexane/acetone, 4:1 (1×) and acetone (2×) for fat]

[b] Major components by TLC is Tioxazafen, does not separate from fat

[c] Residues remaining after exhaustive extractions of liver, or after normal extractions for other samples

na = not applicable

nd = not detected

Characterisation of Radioactive Residue in Liver

Chromatographic characterisation of the soluble ^{14}C-residue from both PH and TH labels showed Tioxazafen (0.002-0.003 mg/kg, 0.3-0.5% TRR) and Tioxazafen Imide (0.009-0.010 mg/kg, 1.4-1.7% TRR) in small amounts, as well as a minor unknown designated Glucuronide conjugate M1 (0.005-0.008 mg/kg, 0.8-1.3% TRR). In addition to these minor metabolites, the PH label liver extract contained benzamidine (0.035 mg/kg, 5.8% TRR) as the only significant extracted residue along with very small amounts of benzamide (0.007 mg/kg, 1.2% TRR). A thiophene-specific very polar residue designated M4 (0.023 mg/kg, 3.5% TRR), which is possibly a mixture of metabolites, was present in the extract of TH-label liver. No single primary residue in liver represented more than 0.035 mg/kg (5.8% TRR).

PES from liver were characterised either by treatment of subsamples with Pronase, a mixture of protease enzymes, or, in an alternative characterisation experiment, sequentially extracting with dilute base and then strong base. The ^{14}C-residues in PES after ACN/water extractions (both PH and TH labels) were solubilized by successive extractions with 0.1 M KOH, followed by 24% KOH. The KOH extracts were acidified and partitioned with ethyl acetate. Residues in the aqueous phase from the 24% KOH extracts of PH label liver contained benzoic acid (0.004 mg/kg, 0.6% TRR), benzamide (0.008 mg/kg, 1.3% TRR) and benzamidine (0.012 mg/kg, 2.0% TRR). Analysis of the EtOAc phase from the 24% KOH extracts of PH label liver showed a significant amount of benzoic acid (0.089 mg/kg, 14.6% TRR). Analysis of the aqueous phase from partitioning of the 24% KOH extracts of TH label liver showed multiple residues, the maximum of which eluted at the solvent front and accounted for 0.040 mg/kg (6.0% TRR) and which likely represented multiple polar residues. The remaining radioactivity corresponded to multiple peaks, none of which corresponded to existing reference standards.

Enzymatic digestion of separate subsamples of PES (both PH and TH labels) with Pronase solubilized 24.7-28.3% of TRR. This suggests that a significant portion of the non-extracted ^{14}C-residues in liver is closely associated with protein (by occlusion, chemical bonding, or extensive metabolism of Tioxazafen to small molecules that are incorporated into constituent amino acids).

Characterisation of Radioactive Residue in Muscle

Small amounts of intact Tioxazafen (0.0003-0.001 mg/kg, 3.7-5.6% TRR) and glucuronide conjugate M1 (0.0002-0.0004 mg/kg, 2.6-4.7% TRR) were detected. Other extracted residues included benzamidine (PH label only, 0.002 mg/kg, 17.5-18.1% TRR) and M4 (TH label only, 0.001-0.002 mg/kg, 11.8-13.2% TRR). The PES contained 0.005-0.010 mg/kg (51.7-66.5% TRR) and was not further analysed.

Characterisation of Radioactive Residue in Fat

87.6-91.6% TRR from the abdominal fat and subcutaneous fat was the initially extracted with acetone/hexane. The extracts were concentrated to remove acetone, and partitioned with ACN/hexane, resulting in 30.6-43.7% TRR in the hexane fraction for the PH-fat and 51.5-55.3% in the hexane fraction for the TH-label fat. TLC analysis showed that the bulk of the radioactivity in the hexane fraction corresponded to tioxazafen, but it was not separable from the lipids in this fraction. Analysis of the acetonitrile

fraction showed the presence of tioxazafen at levels from 12.0-20.7% TRR (0.005-0.010 mg/kg). Even assuming that all of the radioactivity in the hexane fraction was attributed to tioxazafen, the fat would contain a maximum of 0.021-0.034 mg/kg tioxazafen. In the fat from PH-dosed hens, benzonitrile (M3) was identified 21.2–30.1% TRR (0.010-0.013 mg/kg). Further characterisation included hydrolysis of isolated M3 to benzoic acid and analysis by GC/MS.

Characterisation of Radioactive Residue in Eggs

2.9-5.8% TRR (0.002-0.010 mg/kg) from Day 5 AM and Day 8 AM eggs extraction was partitioned in the hexane fraction which, based on TLC analysis, consisted mostly of tioxazafen, although tioxazafen was not separable from the fats in the hexane fraction. The ACN/water phase after hexane partitioning had residues of 0.015-0.028 mg/kg, 15.8-24.1% TRR, which consisted of small amounts of tioxazafen (0.001 mg/kg, 0.8-1.2% TRR), tioxazafen sulfate (0.001-0.002 mg/kg, 1.3-1.4% TRR) and tioxazafen Imide (0.001-0.002 mg/kg, 1.1-1.5% TRR), as well as the minor metabolite glucuronide conjugate M1 (0.003-0.005 mg/kg, 2.6-4.1% TRR). In addition to the sulfate, imide and M1 metabolites, present in both the PH- and TH-label egg extracts, benzamidine (0.003-0.006 mg/kg, 3.4–4.0% TRR) and benzamide (0.001 mg/kg, 0.6% TRR, Day 8 AM only) was detected in PH-label egg extracts. Characterisation of TH-label egg extracts showed a very polar component, designated M4, present at 0.003 mg/kg (1.9% TRR) in Day 8 AM eggs; M4 isolated from liver, where it was found at somewhat higher levels than in eggs, was characterised, but low levels precluded identification. No single primary residue in eggs represented more than 0.006 mg/kg or 4.1% TRR.

Hexane fractions of PH- and TH-label Day 8 AM egg extracts were each partitioned four times with ACN to extract tioxazafen away from fats in the hexane fractions. For each radiolabel, tioxazafen in the combined ACN phases was quantified 0.003-0.004 mg/kg (1.7-2.0% TRR). Thus, the total tioxazafen in Day 8 AM eggs (total in original ACN and hexane fractions) was determined to be 0.005-0.006 mg/kg (3.3% TRR for both radiolabels).

PES residues from eggs were characterised either by treatment of subsamples with protease enzymes (Pronase) or, sequentially extracting with dilute base and then strong base. The PES from Day 8 AM eggs were solubilized by successive extractions with 0.1 M KOH followed by 24% KOH. KOH extracts were separately acidified and partitioned with ethyl acetate. 0.009 and 0.018 mg/kg (5.1 and 12.1% TRR) partitioned into EtOAc after acidification for the TH and PH radiolabels, respectively. The majority of the base-extracted radioactive residue precipitated out of solution upon acidification. Pronase digestion of the PES remaining after extraction of eggs with ACN/water suggested that radioactive residues associated with protein represent 54.4-63.4% TRR. The distribution of the metabolites in eggs is provided in Table 49.

Table 49 Distribution of Radioactive Residues in Eggs from the PH and TH Dose Groups

Compound	PH Label				TH Label			
	Day 5 AM Eggs TRR = 0.086 mg/kg		Day 8 AM Eggs TRR = 0.149 mg/kg		Day 5 AM Eggs TRR = 0.083 mg/kg		Day 8 AM Eggs TRR = 0.175 mg/kg	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Initial Extracts (ACN/water)	27.3	0.023	22.6	0.034	23.1	0.019	21.6	0.038
ACN/water Soluble	24.1	0.021	18.8	0.028	18.5	0.015	15.8	0.028
Tioxazafen	1.2	0.001	0.8	0.001	0.8	0.001	0.8	0.001
Benzamidine	3.4	0.003	4.0	0.006	na	na	na	na
Benzamide	na	na	0.6	0.001	na	na	na	na
Tioxazafen Imide	1.5	0.001	1.5	0.002	1.3	0.001	1.1	0.002
Sulfate Conjugate (M2)	1.3	0.001	1.4	0.002	1.3	0.001	1.4	0.002
Glucuronide Conjugate (M1)	4.0	0.003	2.6	0.004	4.1	0.003	2.6	0.005
Thiophene acid	na	na	na	na	na	na	na	na
M4 (thiophene-specific)	na	na	na	na	na	na	1.9	0.003
Hexane soluble [bc]	2.9	0.002	3.8	0.006	4.6	0.004	5.8	0.010
PES								
KOH treatment	na	na	77.4	0.116	na	na	78.4	0.137
EtOAc Phase	na	na	12.1	0.018	na	na	5.1	0.009
Aqueous phase	na	na	7.0	0.010	na	na	14.3	0.025
Solids-total	na	na	58.3	0.087	na	na	59.0	0.103
Remaining [a]	72.7	0.063	0.0	0.0	76.9	0.064	0.0	0.0

a) Residues remaining after normal extractions, or after KOH extractions when conducted

b) Major component by TLC is Tioxazafen, does not separate from fat (see Footnote 3)

[c] Additional characterisation of Day 8 AM egg hexane fractions (ACN back extraction of hexane fraction and quantitative TLC analysis) indicated Tioxazafen was present in the hexane fractions at 0.003–0.004 mg/kg (1.7–2.0% TRR). Total Tioxazafen in Day 8 AM eggs was 0.005 mg/kg (3.3% TRR, PH label) and 0.006 mg/kg (3.3% TRR, TH label).

na = not applicable

nd = not detected

Characterisation of Radioactive Residue in Excreta

The TRR was 7.069 mg/kg for the PH excreta composite, the combined extract contained 72.6% TRR. HPLC analysis gave numerous poorly resolved peaks. Tioxazafen (0.064 mg/kg, 0.9% %TRR) and benzoic acid (0.177 mg/kg, 2.5% %TRR) were minor components confirmed by TLC; the bulk of the radioactivity in the TLC analysis remained at the origin. PH-label excreta also contained residues which corresponded to benzamide, Tioxazafen Imide, benzamidine (and possibly benzamide oxime). The TRR was 7.985 mg/kg for the TH excreta composite. The combined extract contained 72.1% TRR. HPLC analysis gave numerous poorly resolved peaks, including Tioxazafen (0.8% TRR) and 2-thiophenecarboxylic acid (8.2% TRR), which were both confirmed by TLC, although the resolution for the thiophene acid was poor because of the numerous components in the excreta.

Table 50 Analysis of Residues from [PH-^{14}C]Tioxazafen in Composite Excreta Extract

2278W	Tioxazafen			
Excreta Composite, Phenyl Label	TRR 7.069 mg/kg			
	RT(min)	% HPLC	mg/kg [c]	% of TRR [ab]
Combined AC N:water (1:1) and CAN extracts			5.130	72.6
HPLC				
Tioxazafen region	48.3	1.2	0.064	0.9
M2 region	ca.39.3	ND	NA	NA
M3 (phenyl specific) region	33.3	2.5	0.127	1.8
Tioxazafen Imide region	ca.32.3	2.7	0.141	2
benzoic acid region	ca.29.3	3.5	0.177	2.5
M1 region	ca.25.3	8.1	0.198	2.8
iminoamide region	ca.22	ND	NA	NA
benzamide region	19.3	3.1	0.163	2.3
benzamidine/benzamide oxime region	11.3	10.9	0.58	8.2
max. other single	20.8	11.2	0.573	8.1
PES			1.939	27.4

[a] (extract % TRR) Normalized % TRR from extraction spreadsheet

[b] (residue % TRR) =% HPLC/100 x % TRR (fraction analysed)

[c] mg/kg =% of TRR/100 x TRR

Tioxazafen is extensively metabolized after oral administration to hens, and parent compound is not a major residue in excreta, eggs or tissues, except for fat, where it is present only at very low levels (≤0.034 mg/kg). A proposed metabolic scheme is provided in Figure 10.

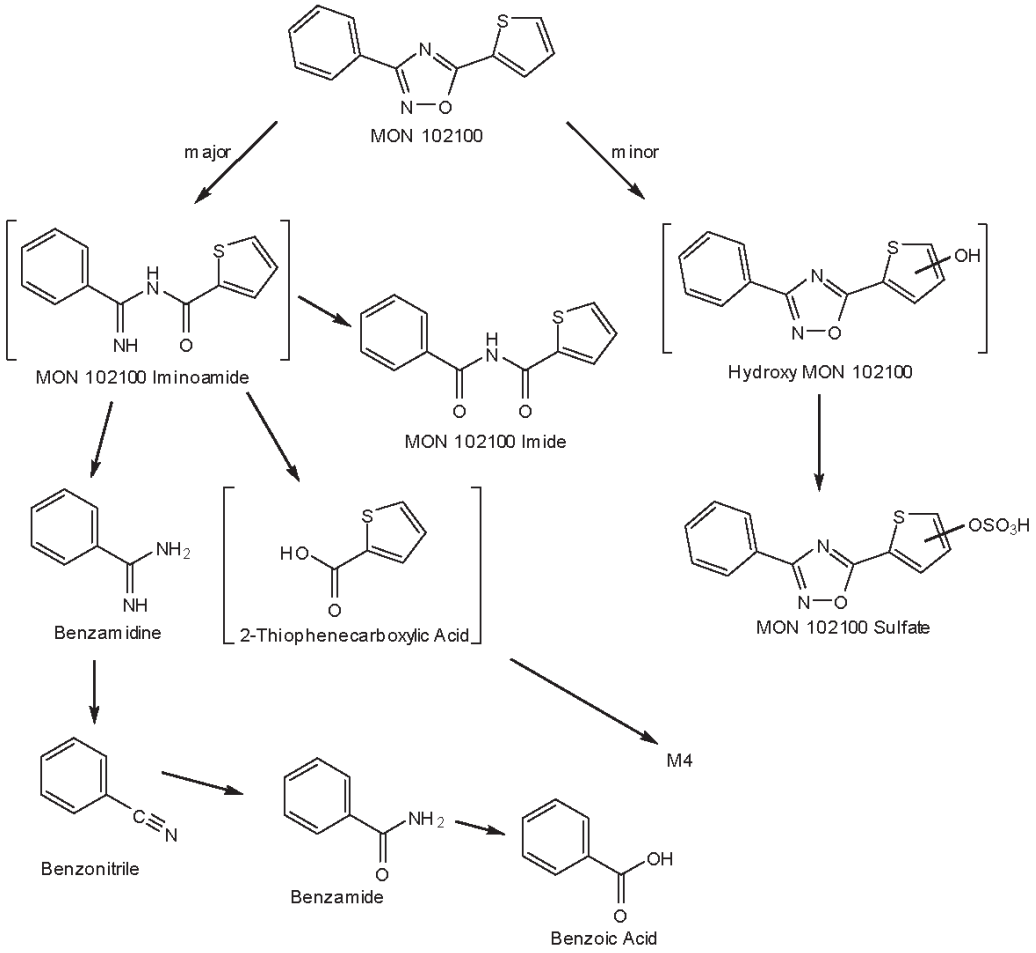


Figure 10 Proposed Metabolic Pathway for Tioxazafen in the Laying Hen

RESIDUE ANALYSIS

The meeting received the information on validation of analysis method for determination of residues of tioxazafen and its metabolites in plant matrices, animal matrices and soil.

Analytical method

The commonly used methods are summarised below:

Method No.	Matrix	Analyte	LOQ (mg/kg)	Method principle
Plant matrices				
EPL 115G806A	soya bean seed, maize grain, soya bean forage, and whole oranges	Tioxazafen benzamidine	0.01 0.01	Extracted with 1:1 acetonitrile/water (v/v) ESI LC-MS/MS analysis
method ME-1604	maize, soya bean, cotton, wheat, sorghum, radish, lettuce, lentils and orange, and processed fractions of maize and soya bean	Tioxazafen	0.0025	Extracted with either toluene or 65% acetonitrile GC-MS/MS analysis
method ME-1579	maize, soya bean, cotton, wheat, sorghum, radish, lettuce, lentils and orange, and processed fractions of maize and soya bean	benzamidine	0.0025	Extracted with either toluene or 65% acetonitrile ESI LC-MS/MS analysis

Method No.	Matrix	Analyte	LOQ (mg/kg)	Method principle
Method ME-1937 (enforcement method)	Maize (grain, forage), soya bean (seed, forage), and orange	Tioxazafen benzamidine	0.005 0.005	Extracted with 65% acetonitrile ESI LC-MS/MS analysis
FDA multi-residue testing methods: Module DG-15		Tioxazafen		GC, flame photometric detector
Animal matrices				
ME-1764, previously identified as AG-ME-1764	milk, fat, liver, kidney, muscle and egg	Tioxazafen benzamidine Benzonitrile 2-thenoylglycine	0.01 0.01 0.025 in fat 0.01 in milk and liver 0.025 in kidney 0.06 in liver	Extracted with ACN for milk, 50% ACN/hexane for fat and 80% ACN in water for all other matrices. EI GC-MS/MS analysis for tioxazafen ESI LC-MS/MS analysis for benzamidine, 2-thenoylglycine and benzonitrile
Soil and water				
AG-ME-1636	soil	Tioxazafen benzamidine	0.005 0.00125	Extracted with 65% acetonitrile EI GC-MS/MS analysis
EPL-BAS 115G761A	drinking, surface and ground water	Tioxazafen benzamidine MON 102130	0.0001 0.0001 0.0001	ESI LC-MS/MS analysis

Method for plant matrices

Analytical Methods Used in Supervised Trials and Processing Studies

EPL 115G806A

An analytical method (EPL 115G806A) was developed and validated for the determination of tioxazafen and benzamidine in crop matrices (Bendler 2014 MSL0025806). The method involves extraction of homogenized samples with 1:1 acetonitrile/water (v/v), supernatant of centrifugation was analysed by ESI LC-MS/MS in positive mode. The limit of quantitation (LOQ) of 0.01 mg/kg for both tioxazafen and benzamidine (as benzamidine per se) were successfully validated and confirmed by independent laboratory validation (Wang 2014b MSL0026072). For benzamidine, the method range of 0.01–0.1 mg/kg and LOQ of 0.01 mg/kg correspond to 0.02–0.2 mg/kg and 0.02 mg/kg in tioxazafen equivalents, respectively.

Average recoveries for soya bean seed, maize grain, soya bean forage, and whole oranges ranged from 94 to 112% for tioxazafen and 85-112% for benzamidine (Tables 51 and 52). The % RSDs ranged from 2 to 13 for tioxazafen and 0-19 for benzamidine. Linearity was shown within 0.01-0.1 mg/kg for tioxazafen and benzamidine with R^2 greater than or equal to 0.996.

Table 51 Recovery data for determination of tioxazafen and benzamidine in crops using method EPL 115G806A

Matrix Type	MS/MS Ion Transitions (*m/z*)	Number of Tests	Fortification Level (mg/kg, mg/kg)	Average Recovery [a] (%)	Standard Deviation	Relative Standard Deviation (%)	
tioxazafen							
Soya bean Seed	Quantitative	5	0.01	107	7	6	
	229.2/111.0	5	0.1	105	13	12	Mean = 9
	Confirmatory	5	0.01	107	13	12	
	229.2/83.0	5	0.1	105	13	13	Mean = 12
Maize Grain	Quantitative	5	0.01	102	1	1	
	229.2/111.0	5	0.1	112	2	1	Mean = 1
	Confirmatory	5	0.01	95	5	5	
	229.2/83.0	5	0.1	112	2	1	Mean = 3
Soya bean Forage	Quantitative	5	0.01	107	4	4	
	229.2/111.0	5	0.1	108	2	2	Mean = 3
	Confirmatory	5	0.01	107	4	3	
	229.2/83.0	5	0.1	107	3	2	Mean = 3
Whole Oranges	Quantitative	5	0.01	94	2	2	
	229.2/111.0	5	0.1	94	2	3	Mean = 2

Matrix Type	MS/MS Ion Transitions (*m/z*)	Number of Tests	Fortification Level (mg/kg, mg/kg)	Average Recovery [a] (%)	Standard Deviation	Relative Standard Deviation (%)
	Confirmatory	5	0.01	99	5	6
	229.2/83.0	5	0.1	97	3	3 Mean = 5
Benzamidine [b]						
Soya bean Seed	Quantitative	5	0.01	106	7	6
	121.0/104.2	5	0.1	109	13	12 Mean = 9
	Confirmatory	5	0.01	112	19	17
	121.0/77.1	5	0.1	108	15	14 Mean = 16
Maize Grain	Quantitative	5	0.01	98	2	2
	121.0/104.2	5	0.1	105	1	1 Mean = 2
	Confirmatory	5	0.01	92	9	10
	121.0/77.1	5	0.1	104	1	1 Mean = 6
Soya bean Forage	Quantitative	5	0.01	93	4	4
	121.0/104.2	5	0.1	104	3	2 Mean = 3
	Confirmatory	5	0.01	89	9	10
	121.0/77.1	5	0.1	97	2	3 Mean = 7
Whole Oranges	Quantitative	5	0.01	85	0	0
	121.0/104.2	5	0.1	85	0	0 Mean = 0
	Confirmatory	5	0.01	96	2	2
	121.0/77.1	5	0.1	86	1	1 Mean = 2

[a] Individual recoveries met the 70-120% acceptance criterion except one soya bean seed sample (142%) fortified with benzamidine using the confirmatory ion at the 0.01 mg/kg (mg/kg) fortification level.

[b] Benzamidine is reported as benzamidine per se. Benzamidine concentrations of 0.01 and 0.1 mg/kg (mg/kg) correspond to 0.02 and 0.2 mg/kg (mg/kg) in tioxazafen equivalents.

Table 52 Independent laboratory recovery data for determination of tioxazafen and benzamidine in crops using method EPL 115G806A

Compound	Matrix Type	Fortification Level (mg/kg)	Number of Tests	Average Recovery[a] (%)	Standard Deviation	RSD (%)
tioxazafen	Soya bean seed	0.01	5	96	12	12
		0.1	5	112	6	5
			Total =10			Mean = 9
	Soya bean forage	0.01	5	105	8	8
		0.1	5	108	5	5
			Total =10			Mean = 6
Benzamidine [b]	Soya bean seed	0.01	5	114	4	3
		0.1	5	118	4	3
			Total =10			Mean = 3
	Soya bean forage	0.01	5	115	5	4
		0.1	5	119	4	3
			Total =10			Mean = 4

[a] Individual recoveries met the 70-120% acceptance criterion except one soya bean forage sample (124%) and two soya bean seed samples (121% and 122%) fortified with benzamidine at the fortification level of 0.1 mg/kg, mg/kg.

[b] Benzamidine is reported as benzamidine per se. Benzamidine concentrations of 0.01 and 0.1 mg/kg (mg/kg) correspond to 0.02 and 0.2 mg/kg (mg/kg) in tioxazafen equivalents.

Method ME-1604 and method ME-1579

Analytical methods were developed and validated for the determination of tioxazafen (method ME-1604) and its major plant metabolite, benzamidine (method ME-1579) in raw agricultural commodities of maize, soya bean, cotton, wheat, sorghum, radish, lettuce, lentils and orange, and processed fractions of maize and soya bean (Riter *et al*., 2014 MSL0025804). The tioxazafen analytical method involves the extraction of homogenized raw or processed agricultural commodities with either toluene or 65% acetonitrile in water containing stable isotopically-labelled tioxazafen internal standard. Samples extracted (extracted using 65% ACN in water are partitioned into toluene) are analysed by GC-MS/MS after centrifugation. The benzamidine method uses 50%

acetonitrile in water containing the stable-label benzamidine internal standard for extraction. A portion of this extract is diluted 15-fold with 95% acetonitrile in 10 mM ammonium formate to adjust the sample composition for hydrophilic interaction chromatography (HILIC) and analysis by ESI LC-MS/MS. The LOQ was 0.0025 mg/kg for both tioxazafen and benzamidine (in tioxazafen equivalents) for all matrices tested.

Tables 53 to 59. Average recoveries ranged from 80 to 114% for tioxazafen and 83% to 115% for benzamidine. The LOQs were 0.0025 mg/kg for tioxazafen and 0.0025 mg/kg for benzamidine in maize, soya bean and cotton. The %RSDs ranged from 2.1 to 10.1 for tioxazafen and 2.0 to 12.5 for benzamidine. Linearity was good for injections between 0.0025 and 0.1 µg/mL (r2 ≥0.99xx).

Table 53 Recovery data for tioxazafen using method ME-1604

Matrix Type	Fortification Level [a] (mg/kg)	Number of Tests	Average Recovery (%)[e]	Standard Deviation	Relative Standard Deviation (%)
Maize grain	0.010 0.10	6 6 Total = 12	90 100	3.5 1.5	3.9 1.5 Mean = 2.7
Maize stover	0.010 0.10	6 6 Total = 12	101 104	6.8 3.3	6.7 3.2 Mean = 4.9
Maize forage	0.010 0.10	6 6 Total = 12	88 96	3.4 3.2	3.9 3.3 Mean = 3.6
Maize processed fractions[b]	0.010 0.10	15 15 Total = 30	95 99	5.1 1.8	5.4 1.8 Mean = 3.6
Soya bean seed	0.0025 0.010 0.10	6 6 6 Total = 18	111 104 100	7.8 3.5 1.8	7.0 3.4 1.8 Mean = 4.1
Soya bean Seed[c]	0.00125 0.005 0.05	6 6 6 Total = 18	103 93 91	15.7 5.4 2.5	15.3 5.8 2.7 Mean = 7.9
Soya bean Seed[d]	0.0025 0.010 0.10	5 6 6 Total = 17	86 96 102	11.1 7.1 2.0	12.9 7.3 2.0 Mean = 7.4
Cotton seed	0.0025 0.010 0.10	6 6 6 Total = 18	97 100 100	4.1 2.3 1.3	4.3 2.3 1.3 Mean = 2.6
Orange	0.0025 0.010 0.10	6 6 6 Total = 18	103 102 100	1.6 9.0 2.7	1.6 8.9 2.7 Mean = 4.4
Radish	0.0025 0.010 0.10	6 6 6 Total = 18	96 98 100	9.8 4.8 2.6	10.2 4.9 2.6 Mean = 5.9
Lentils	0.0025 0.010 0.10	6 6 6 Total = 18	100 98 99	16.4 4.8 8.9	16.3 4.9 9.0 Mean = 10.1
Lentils[c]	0.00125 0.005 0.05	6 6 6 Total = 18	99 98 103	7.3 3.0 3.4	7.4 3.1 3.3 Mean = 4.6

[a] Fortifications were performed with tioxazafen reference standard solutions.

[b] Processed fractions include oil, meal, flour, starch, and grits.

[c] Results with extraction procedure using 65% ACN in water followed by evaporation and reconstitution in toluene. This procedure has been superseded by direct partitioning into toluene without evaporation.

[d] Results with extraction procedure using 65% ACN in water followed by partitioning into toluene.

[e] Individual recoveries met a 70-120% recovery acceptance criterion.

Table 54 Recovery data for benzamidine (as tioxazafen Equivalents) determined using method ME-1579

Matrix Type	Fortification Level[a] (mg/kg)	Number of Tests	Average Recovery (%)[c]	Standard Deviation	Relative Standard Deviation (%)
Maize grain	0.010 0.10	6 6 Total = 12	109 110	2.1 1.8	1.9 1.6 Mean = 1.8
Maize stover	0.010 0.10	6 6 Total = 12	109 108	1.8 1.9	1.6 1.7 Mean = 1.7
Maize forage	0.010 0.10	6 6 Total = 12	107 108	2.5 1.5	2.3 1.4 Mean = 1.8
Maize processed fractions [b]	0.010 0.10	15 15 Total = 30	109 108	5.1 3.9	4.7 3.6 Mean = 4.1
Soya bean seed	0.0025 0.010 0.10	6 6 6 Total = 18	97 104 107	2.5 1.8 1.5	2.5 1.7 1.4 Mean = 1.9
Cotton seed	0.0025 0.010 0.10	6 6 6 Total = 18	104 109 110	1.2 3.2 2.2	1.2 2.9 2.0 Mean = 2.1
Orange	0.0025 0.010 0.10	6 6 6 Total = 18	114 114 90	2.6 3.6 1.8	2.3 3.2 2.0 Mean = 2.5
Radish	0.0025 0.010 0.10	6 6 6 Total = 18	99 109 95	5.1 3.2 4.0	5.1 2.9 4.2 Mean = 4.1
Lentils	0.0025 0.010 0.10	5 5 5 Total = 15	80 102 100	2.4 3.3 6.0	2.9 3.3 6.0 Mean = 4.1

[a] Fortifications were performed with benzamidine reference standard solutions.

[b] Maize processed fractions included: oil, flour, grits, starch and meal.

[c] Individual recoveries met a 70-120% recovery acceptance criterion.

Table 55 In-Study Validation Recovery Results for tioxazafen for Maize, Soya bean and Cotton in Crop Residue Field Trials using method ME-1604

Matrix Type[a]	Fortification Level[b] (mg/kg, mg/kg)	Number of Tests	Average Recovery (%)[e]	Standard Deviation	Relative Standard Deviation (%)
Maize grain	0.0025 0.010 0.10	18 18 18 Total = 54	93 96 98	10.2 7.4 3.9	10.9 7.7 4.0 Mean = 7.6
Maize stover	0.0025 0.010 0.10	9 9 9 Total = 27	97 98 97	7.9 7.8 3.9	8.2 7.9 4.0 Mean = 6.7
Maize forage	0.0025 0.010 0.10	9 9 9 Total = 27	98 98 100	12.0 5.7 2.5	12.1 5.8 2.5 Mean = 6.8

Matrix Type[a]	Fortification Level[b] (mg/kg, mg/kg)	Number of Tests	Average Recovery (%)[e]	Standard Deviation	Relative Standard Deviation (%)
Maize processed fractions[c]	0.0025 0.010 0.10	14 7 7 Total = 28	91 95 93	11.8 9.6 3.2	12.9 10.1 3.4 Mean = 8.8
Soya bean seed	0.0025 0.010 0.10	12 12 12 Total = 36	94 99 99	9.7 4.7 1.8	10.3 4.7 1.8 Mean = 5.6
Soya bean processed fractions[d]	0.0025 0.010 0.10	12 12 12 Total = 36	101 98 97	11.6 6.2 2.3	11.5 6.3 2.4 Mean = 6.7
Soya bean forage	0.0025 0.010 0.10	11 12 12 Total = 35	98 101 97	12.3 3.4 2.8	12.6 3.4 2.9 Mean = 6.3
Soya bean hay	0.0025 0.010 0.10	18 18 18 Total = 54	94 97 98	9.4 5.7 2.3	10.0 5.9 2.3 Mean = 6.1
Cotton seed	0.0025 0.010 0.10	8 8 6 Total = 22	100 100 96	15.0 7.0 2.5	15.1 7.0 2.6 Mean = 8.2
Cotton gin by-products	0.0025 0.010 0.10	3 3 3 Total = 9	91 103 100	9.1 1.9 2.7	10.0 1.8 2.7 Mean = 4.8

[a] From maize (Mueth, 2014a), soya bean (Mueth, 2014b), and cotton (Mueth, 2014c) residue field trials.

[b] Fortifications were performed with tioxazafen reference standard solutions.

[c] Processed fractions include oil, meal, flour, starch, and grits.

[d] Processed fractions include hull, oil, meal, and lecithin.

[e] Individual recoveries met a 70-120% recovery acceptance criterion except two cotton seed samples fortified with tioxazafen at 0.0025 mg/kg which had recoveries of 66.8% and 69.6%.

Table 56 In-Study Validation Recovery Results for Benzamidine (as tioxazafen Equivalents) for Maize, Soya bean and Cotton in Crop Residue Field Trials using method ME-1579

Matrix Type [a]	Fortification Level [b] (mg/kg)	Number of Tests	Average Recovery (%)[e]	Standard Deviation	Relative Standard Deviation (%)
Maize grain	0.0025 0.010 0.10	9 9 9 Total = 27	102 102 105	4.7 2.3 3.6	4.6 2.3 3.5 Mean = 3.4
Maize stover	0.0025 0.010 0.10	9 9 9 Total = 27	99 105 107	3.7 2.4 2.3	3.8 2.3 2.1 Mean = 2.7
Maize forage	0.0025 0.010 0.10	12 12 12 Total = 36	99 102 106	4.8 2.5 2.0	4.8 2.4 1.9 Mean = 3.0
Maize processed fractions [c]	0.0025 0.010 0.10	14 7 7 Total = 28	85 102 106	3.3 1.3 1.1	3.8 1.3 1.0 Mean = 2.0

Matrix Type [a]	Fortification Level [b] (mg/kg)	Number of Tests	Average Recovery (%)[e]	Standard Deviation	Relative Standard Deviation (%)
Soya bean seed	0.0025 0.010 0.10	14 15 15 Total = 44	94 98 105	6.3 2.9 1.6	6.7 2.9 1.5 Mean = 3.7
Soya bean processed fractions [d]	0.0025 0.010 0.10	11 12 12 Total = 35	101 96 105	10.5 8.2 1.9	10.5 8.5 1.9 Mean = 7.0
Soya bean forage	0.0025 0.010 0.10	14 14 14 Total = 42	95 101 105	8.0 4.7 3.1	8.4 4.6 3.0 Mean = 5.3
Soya bean hay	0.0025 0.010 0.10	14 14 13 Total = 41	96 101 105	4.4 5.1 2.8	4.6 5.1 2.7 Mean = 4.1
Cotton seed	0.0025 0.010 0.10	9 9 9 Total = 27	83 86 83	8.8 7.6 2.5	10.6 8.8 3.0 Mean = 7.5
Cotton gin by-products	0.0025 0.010 0.10	3 3 3 Total = 9	93 91 94	10.4 3.6 3.0	11.3 4.0 3.2 Mean = 6.1

[a] From maize (Mueth, 2014a), soya bean (Mueth, 2014b), and cotton (Mueth, 2014c) residue field trials.

[b] Fortifications were performed with benzamidine reference standard solutions.

[c] Processed fractions include oil, meal, flour, starch, and grits.

[d] Processed fractions include hulls, oil, meal, and lecithin.

[e] Individual recoveries met a 70-120% recovery acceptance criterion.

Table 57 In-Study Validation Recovery Results for tioxazafen for Lettuce, Radish, Wheat and Sorghum in a Limited Field Rotational Crop Study using method ME-1604

Matrix Type [a]	Fortification Level [b] (mg/kg, mg/kg)	Number of Tests	Average Recovery (%)[c]	Standard Deviation	Relative Standard Deviation (%)
Wheat forage	0.0025 0.010 0.10	3 3 2 Total = 8	96 83 96	12.4 0.9 2.8	12.9 1.1 2.9 Mean = 5.6
Wheat grain	0.0025 0.010 0.10	3 3 3 Total = 9	97 85 102	2.6 2.6 0.6	2.7 3.1 0.6 Mean = 2.1
Wheat hay	0.0025 0.010 0.10	3 3 3 Total = 9	94 92 92	13.3 0.7 2.5	14.1 0.8 2.7 Mean = 5.9
Wheat straw	0.0025 0.010 0.10	3 3 3 Total = 9	86 92 91	3.2 1.8 3.0	3.7 2.0 3.3 Mean = 3.0
Radish tops	0.0025 0.010 0.10	6 6 6 Total = 18	97 105 100	12.3 2.9 4.1	12.7 2.8 4.0 Mean = 6.5

Matrix Type [a]	Fortification Level [b] (mg/kg, mg/kg)	Number of Tests	Average Recovery (%)[c]	Standard Deviation	Relative Standard Deviation (%)
Radish roots	0.0025 0.010 0.10	5 6 6 Total = 17	101 104 101	7.4 3.7 4.2	7.3 3.5 4.1 Mean = 5.0
Sorghum forage	0.0025 0.010 0.10	3 3 3 Total = 9	88 102 99	3.7 3.3 1.5	4.2 3.3 1.6 Mean = 3.0
Sorghum grain	0.0025 0.010 0.10	3 3 3 Total = 9	93 97 98	12.7 7.0 1.4	13.6 7.2 1.4 Mean = 7.4
Sorghum stover	0.0025 0.010 0.10	3 3 3 Total = 9	100 98 96	4.5 3.3 1.1	4.5 3.4 1.1 Mean = 3.0
Lettuce	0.0025 0.010 0.10	6 6 6 Total = 18	111 103 101	3.7 2.3 4.1	3.3 2.2 4.0 Mean = 3.2

[a] From limited field rotational crop residue study (Urbanczyk-Wochniak and Riter, 2014).

[b] Fortifications were performed with tioxazafen reference standard solutions.

[c] Individual recoveries met a 70-120% recovery acceptance criterion.

Table 58 In-Study Validation Recovery Results for Benzamidine (as tioxazafen Equivalents) for Lettuce, Radish, Wheat and Sorghum in a Limited Field Rotational Crop Study using method ME-1579

Matrix Type [a]	Fortification Level [b] (mg/kg)	Number of Tests	Average Recovery (%)[c]	Standard Deviation	Relative Standard Deviation (%)
Lettuce	0.0025 0.010 0.10	6 6 6 Total = 18	84 91 91	3.6 11.7 11.6	4.3 12.9 12.8 Mean = 10.0
Radish tops	0.0025 0.010 0.10	6 6 6 Total = 18	95 91 91	13.2 9.9 11.6	13.9 10.9 12.8 Mean = 12.5
Radish roots	0.0025 0.010 0.10	6 6 6 Total = 18	91 89 90	8.2 8.9 10.0	8.9 10.0 11.1 Mean = 10.0
Sorghum forage	0.0025 0.010 0.10	3 3 3 Total = 9	88 97 102	2.9 1.7 1.6	3.3 1.8 1.6 Mean = 2.2
Sorghum grain	0.0025 0.010 0.10	3 3 3 Total = 9	84 97 101	1.8 1.8 0.8	2.2 1.8 0.7 Mean = 1.6
Sorghum stover	0.0025 0.010 0.10	3 3 3 Total = 9	92 98 101	2.4 1.0 0.9	2.6 1.0 0.9 Mean = 1.5
Wheat forage	0.0025 0.010 0.10	3 3 3	99 110 111	3.6 1.7 1.5	3.6 1.6 1.4

Matrix Type [a]	Fortification Level [b] (mg/kg)	Number of Tests	Average Recovery (%)[c]	Standard Deviation	Relative Standard Deviation (%)
		Total = 9			Mean = 2.2
Wheat grain	0.0025 0.010 0.10	3 3 3 Total = 9	83 106 110	2.1 3.5 3.1	2.5 3.3 2.8 Mean = 2.9
Wheat hay	0.0025 0.010 0.10	3 3 3 Total = 9	107 109 116	0.7 0.0 3.1	0.6 0.0 2.6 Mean = 1.1
Wheat straw	0.0025 0.010 0.10	3 3 3 Total = 9	87 106 115	4.8 4.5 1.5	5.5 4.2 1.3 Mean = 3.7

[a] From limited field rotational crop residue study (Urbanczyk-Wochniak and Riter, 2014;).

[b] Fortifications were performed with benzamidine reference standard solutions.

[c] Individual recoveries met a 70-120% recovery acceptance criterion.

Radiovalidation of the extraction step using toluene/water for crop matrices

A comparison of the extractability of residues in soy bean hay, maize stover and soy bean seed by acetone/H_2O versus toluene/H_2O extraction showed that ^{14}C-tioxazafen and ^{14}C-benzamidine could be recovered equally well with the solvent system used in metabolism studies (acetone/H_2O) and in the analytical method (ACN/H2O).

Table 59 Comparison of ^{14}C extracted using acetone/H2O versus toluene/H2O

Matrix	Extraction Solvent	tioxazafen		Benzamidine	
		%TRR (mg/kg)	Relative Extraction Efficiency (%)*	%TRR (mg/kg)	Relative Extraction Efficiency (%)*
Maize Thinnings	Acetone/H_2O	33.1 (0.569)		NA	
	Toluene/H_2O	22.0 (0.364)	66	NA	
Soya bean Hay	Acetone/H_2O	4.33 (0.034)		8.11 (0.063)	
	ACN/H_2O	3.83 (0.029)	88	8.86 (0.068)	109
	Toluene/H_2O	4.39 (0.033)	101	NA	
Maize Stover	Acetone/H_2O	NA		11.2 (0.007)	
	ACN/H_2O	NA		11.0 (0.007)	98
Soya bean Seed	Acetone/H_2O	NA		10.9 (0.008)	
	ACN/H_2O	NA		9.21 (0.006)	85

* Efficiency of the residue method extraction procedure compared to the metabolism study procedure for the specific analyte (tioxazafen or benzamidine)

NA = not applicable for the purposes of this evaluation

Method ME-1937 (Enforcement method)

An analytical enforcement method was developed (Riter *et al*., 2017 MSL0027188) and validated (Sharp 2015 MSL0027228) for the determination of tioxazafen and its major plant metabolite, benzamidine, in raw agricultural commodities of maize, soya bean, and orange. The method involves the extraction of homogenized raw agricultural commodities with 65% acetonitrile in water containing stable-label tioxazafen and benzamidine internal standards, and analysis by ESI LC-MS/MS. The limit of quantitation (LOQ) is 0.0050 mg/kg for both tioxazafen and benzamidine (in tioxazafen equivalents) for all matrices tested.

Accuracy and precision results for tioxazafen and benzamidine for each crop type evaluated during the method validation phase are summarised in Tables 60 and 61.

Table 60 Validation Recovery Results for tioxazafen using method ME-1937

Matrix Type	Fortification Level (mg/kg)	Number of Tests	Average Recovery (%)	Standard Deviation	Relative Standard Deviation (%)
Maize grain	0.005 0.05	6 6 Total = 12	98 101	4.2 1.8	4.3 1.8 Mean = 3.0
Maize forage	0.005 0.05	6 6 Total = 12	98 98	9.0 4.6	9.2 4.7 Mean = 7.0
Orange	0.005 0.05	6 6 Total = 12	97 99	6.2 4.0	6.4 4.0 Mean = 5.2
Soya bean seed	0.005 0.05	6 6 Total = 12	103 100	9.4 3.5	9.1 3.5 Mean = 6.3

Table 61 Validation Recovery Results for Benzamidine (as tioxazafen equivalents) using method ME-1937

Matrix Type	Fortification Level (mg/kg)	Number of Tests	Average Recovery (%)	Standard Deviation	Relative Standard Deviation (%)
Maize grain	0.005 0.05	6 6 Total = 12	88 104	3.8 2.1	4.4 2.0 Mean = 3.2
Maize forage	0.005 0.05	6 6 Total = 12	106 100	6.1 3.0	5.7 3.0 Mean = 4.4
Orange	0.005 0.05	6 6 Total = 12	102 100	3.7 1.1	3.6 1.1 Mean = 6.3
Soya bean Seed	0.005 0.05	6 6 Total = 12	106 106	3.6 1.6	3.4 1.5 Mean = 5.2

The average recoveries of tioxazafen and benzamidine, using the primary quantitation ion transition, at both fortification levels in both matrices tested (soya bean forage and soya bean seed) were within the range of 70 to 120%. The relative standard deviations (RSDs) of replicate measurements were ≤ 20% for both analytes at each fortification level in each matrix. The results obtained are summarised in Table 62.

Table 62 Recovery Results Obtained by an Independent Laboratory for the Determination of tioxazafen and Benzamidine (as tioxazafen equivalents) in Crop Matrices using method ME-1937

Compound	Matrix Type	Fortification Level (mg/kg, mg/kg)	No. of Tests	Average Recovery* (%)	SD	RSD (%)
tioxazafen	Soya bean Seed	0.005 0.05	5 5 Total = 10	100 96	11 3	11 3 Mean = 7
	Soya bean Forage	0.005 0.05	5 5 Total = 10	101 98	20 4	19 4 Mean = 11.5
Benzamidine	Soya bean Seed	0.005 0.05	5 5 Total = 10	79 71	2 3	3 4 Mean = 3.5
	Soya bean Forage	0.005 0.05	5 5 Total = 10	91 91	2 2	2 2 Mean = 2

Fortifications were performed with benzamidine or tioxazafen reference standard solutions

* Individual recoveries met the 70-120% acceptance criterion except for one soya bean forage sample (128%) fortified with tioxazafen at 0.005 mg/kg (mg/kg) and one soya bean seed sample (69%) fortified with benzamidine at the fortification level of 0.05 mg/kg (mg/kg).

Radiovalidation of the extraction step in using acetonitrile/water for crop matrices

A comparison of the extractability of residues in soy bean hay, maize stover and soy bean seed by acetone/H_2O versus acetonitrile/H_2O extraction showed that ^{14}C-tioxazafen and ^{14}C-benzamidine could be recovered equally well with the solvent system used in metabolism studies (acetone/H2O) and in the analytical method (acetonitrile/H_2O).The results are summarised in the Table 63. ACN/H2O extracted same benzamidine to Acetone/H_2O. However, more than twice tioxazafen was extracted with ACN/H_2O.

Table 63 Comparison of Recovery Results Obtained in Endogenous Method Validation versus the Metabolism Study

Matrix	Extraction Solvent	tioxazafen		Benzamidine	
		% TRR (mg/kg)	Relative Extraction Efficiency (%)*	% TRR (mg/kg)	Relative Extraction Efficiency (%)*
Soya bean Hay	Acetone/H_2O	3.4 (0.026)	253	8.4 (0.063)	100
	ACN/H_2O	8.6 (0.064)		8.4 (0.063)	

*Efficiency of the residue method extraction procedure compared to the metabolism study procedure

FDA multiresidue testing methods (MRMs)

The analytical method for determination of tioxazafen (Sears 2014 MSL0025544) and benzamidine (Sears 2014 MSL0025807) were evaluated according to the FDA multiresidue testing methods (MRMs) in Appendix II of the Pesticide Analytical Manual, Volume I (PAM I), third edition. GLC module DG 15 was found to be adequate for the determination of tioxazafen however a suitable protocol for benzamidine was not found.

Method for food of animal origin

An analytical method (ME-1764, previously identified as AG-ME-1764) was developed (Huang *et al*., 2014 MSL0025799) and validated (Sharp 2014 MSL0025800) 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 homogenized samples with extraction solution containing stable isotopically-labelled internal standards for tioxazafen, benzamidine, 2-thenoylglycine and/or benzonitrile. The composition of the extraction solution is ACN for milk, 50% ACN/hexane for fat and 80% ACN in water for all other matrices.

For fat, a portion of the extract from the ACN layer is transferred for analysis of tioxazafen and/or benzonitrile by EI GC-MS/MS. Water is added to the remaining extraction mixture and it is shaken and centrifuged again. Another portion of the extract from the ACN/water layer is transferred and diluted with an equal volume of ACN for analysis of benzamidine by ESI LC-MS/MS.

For all other animal matrices, two aliquots of the extract are transferred for further processing and analysis. One aliquot is directly partitioned with toluene and a portion of the toluene phase is transferred for analysis of tioxazafen by EI GC-MS/MS. For the other aliquot, samples extracted using 80% ACN in water are diluted with an equal volume of ACN before analysis, whereas those extracted in ACN are directly analysed by ESI LC-MS/MS for benzamidine and/or 2-thenoylglycine.

The limit of quantitation (LOQ) for tioxazafen and benzamidine (as tioxazafen equivalents) was determined to be 0.010 mg/kg (mg/kg) in all matrices. The LOQ for benzonitrile (as tioxazafen equivalents) was determined to be 0.025 mg/kg (mg/kg) in fat, and for 2-thenoylglycine (as tioxazafen equivalents) was determined to be 0.010 mg/kg (mg/kg) for milk and liver, and 0.025 mg/kg for kidney. The LOQ for 2-thenoylglycine (as tioxazafen equivalents) in liver was modified to 0.06 mg/kg during in-study validation due to matrix interferences.

The analytical method ME-1764 was successfully validated by an independent laboratory for tioxazafen and benzamidine in four representative animal matrices (bovine kidney, bovine muscle, milk and egg)

The average recoveries for tioxazafen and its metabolites benzamidine, 2-thenoylglycine and benzonitrile are summarised in Tables 64 through to Table 69.

Table 64 Pre-Study Validation Recovery Results for tioxazafen

Matrix Type	Fortification Level [a] (mg/kg, mg/kg)	Number of Tests	Average Recovery (%)[b]	Standard Deviation	Relative Standard Deviation (%)
Milk	0.010	6	103	1.4	1.3
	0.10	6	96	2.3	2.4
	1.2	6	100	1.6	1.6

Matrix Type	Fortification Level [a] (mg/kg, mg/kg)	Number of Tests	Average Recovery (%)[b]	Standard Deviation	Relative Standard Deviation (%)
		Total = 18			Mean = 1.8
Liver	0.010 0.10 1.2	6 6 6 Total = 18	92 93 93	9.5 5.9 7.5	10.3 6.3 8.0 Mean = 8.2
Kidney	0.010 0.10 1.2	6 6 6 Total = 18	104 99 103	7.5 1.9 1.1	7.3 1.9 1.1 Mean = 3.4
Muscle	0.010 0.10 1.2	6 6 6 Total = 18	103 104 103	8.7 6.3 1.2	8.5 6.1 1.1 Mean = 5.2
Fat	0.010 0.10 1.2	5 6 6 Total = 17	96 98 100	2.0 0.9 0.9	2.1 0.8 0.9 Mean = 1.3
Egg	0.010 0.10 1.2	6 6 6 Total = 18	105 97 99	5.1 1.7 0.6	4.8 1.7 0.6 Mean = 2.4

[a] Fortifications were performed with tioxazafen reference standard solutions.

[b] Individual recoveries met a 70-120% recovery acceptance criterion.

Table 65 Pre-Study Validation Recovery Results for Benzamidine (as tioxazafen Equivalents)

Matrix Type	Fortification Level [a] (mg/kg)	Number of Tests	Average Recovery (%)[b]	Standard Deviation	Relative Standard Deviation (%)
Milk	0.010 0.10 1.2	6 6 6 Total = 18	103 108 95	2.2 2.9 1.7	2.2 2.7 1.8 Mean = 2.2
Liver	0.010 0.10 1.2	6 6 6 Total = 18	105 106 101	3.7 1.8 2.1	3.5 1.7 2.1 Mean = 2.4
Kidney	0.010 0.10 1.2	6 6 6 Total = 17	104 104 100	2.4 1.5 2.8	2.3 1.5 2.8 Mean = 2.2
Muscle	0.010 0.10 1.2	6 6 6 Total = 18	98 101 100	2.6 1.7 2.3	2.6 1.7 2.3 Mean = 2.2
Fat	0.010 0.10 1.2	6 6 6 Total = 18	99 106 93	0.7 2.7 0.9	0.7 2.6 0.9 Mean =1.4
Egg	0.010 0.10 1.2	6 6 6 Total = 18	96 98 93	2.2 0.7 2.1	2.3 0.7 2.2 Mean = 1.7

[a] Fortifications were performed with tioxazafen reference standard solutions.

[b] Individual recoveries met a 70-120% recovery acceptance criterion.

Table 66 Pre-Study Validation Recovery Results for 2-Thenoylglycine (as tioxazafen Equivalents)

Matrix Type	Fortification Level[a] (mg/kg, mg/kg)	Number of Tests	Average Recovery (%)[b]	Standard Deviation	Relative Standard Deviation (%)
Milk	0.010	6	97	1.2	1.2
	0.10	6	108	4.6	4.3
	1.2	6	105	4.9	4.7
		Total = 18			Mean =3.4
Liver	0.010	6	84	2.6	3.1
	0.10	6	91	4.0	4.4
	1.2	6	84	1.5	1.8
		Total = 18			Mean =3.1
Kidney	0.025	6	98	8.5	8.7
	0.10	6	100	9.4	9.4
	1.2	6	94	4.7	4.9
		Total = 18			Mean =7.7

[a] Fortifications were performed with tioxazafen reference standard solutions.

[b] Individual recoveries met a 70-120% recovery acceptance criterion.

Table 67 Pre-Study Validation Recovery Results for Benzonitrile (as tioxazafen Equivalents)

Matrix Type	Fortification Level[a] (mg/kg, mg/kg)	Number of Tests	Average Recovery (%)[b]	Standard Deviation	Relative Standard Deviation (%)
Fat	0.025	5	89	2.6	2.9
	0.10	6	93	0.9	0.9
	1.2	6	96	1.1	1.2
		Total = 17			Mean =1.7

[a] Fortifications were performed with tioxazafen reference standard solutions.

[b] Individual recoveries met a 70-120% recovery acceptance criterion.

Table 68 In-Study Validation Recovery Results for tioxazafen and Metabolites in Milk, Fat, Liver, Kidney, and Muscle from Cattle

Matrix Type[a]	Analyte	Fortification Level[b] (mg/kg, mg/kg)	Number of Tests	Average Recovery (%)[c]	Standard Deviation	Relative Standard Deviation (%)
Milk	tioxazafen	0.010	10	95.5	11.4	11.9
		0.10	6	97.2	3.5	3.6
	Benzamidine	0.010	17	94.8	9.1	9.6
		0.10	10	98.3	7.4	7.5
	2-Thenoylglycine	0.010	13	94	9.5	10.1
		0.10	8	94.8	10.3	10.9
Fat	tioxazafen	0.010	7	98	5.7	5.8
		0.10	3	97.3	1.2	1.2
	Benzamidine	0.010	9	93.7	2.9	3.1
		0.10	5	87.4	5.4	6.1
	Benzonitrile	0.025	7	92.3	4.1	4.5
		0.25	3	93	2.6	2.8
Liver	tioxazafen	0.010	7	99.4	7.3	7.3
		0.10	3	101.3	2.5	2.5
	Benzamidine	0.010	8	105.3	14.6	13.9
		0.10	3	101.7	1.5	1.5
		0.25	3	103.3	0.6	0.6
	2-Thenoylglycine	0.060	7	95.7	5.4	5.6

Matrix Type[a]	Analyte	Fortification Level[b] (mg/kg, mg/kg)	Number of Tests	Average Recovery (%)[c]	Standard Deviation	Relative Standard Deviation (%)
		0.25	3	94.3	7.8	8.2
Kidney	tioxazafen	0.010	7	94.9	5.3	5.6
		0.10	3	93.7	2.1	2.2
	Benzamidine	0.010	7	84.3	6.5	7.7
		0.10	3	100.7	0.6	0.6
		0.25	3	99.7	1.5	1.5
	2-Thenoylglycine	0.025	7	77.7	6	7.8
		0.25	3	104	1	1
Muscle	tioxazafen	0.010	7	95.6	4	4.2
		0.10	3	98.3	2.5	2.6
	Benzamidine	0.010	7	79.1	3.8	4.9
		0.10	3	107.3	1.5	1.4

[a] From cattle feeding study (Brungardt, 2014)

[b] Fortifications were performed with tioxazafen, benzamidine, 2-thenoylglycine and benzonitrile reference standard solutions.

[c] Individual recoveries met the 70-120% acceptance criterion except for one milk sample (67%) fortified with 2-thenoylglycine at the fortification level of 0.01 mg/kg (mg/kg).

Table 69 In-Study Validation Recovery Results for tioxazafen and Metabolites in Eggs, Fat, Liver, and Muscle from Hens

Matrix Type[a]	Analyte	Fortification Level[b] (mg/kg)	Number of Tests	Average Recovery (%)[c]	Standard Deviation	Relative Standard Deviation (%)
Egg	tioxazafen	0.010	13	96.6	13.2	13.7
		0.10	8	98.5	5.0	5.1
	Benzamidine	0.010	14	88.0	13.4	15.3
		0.10	9	94.7	2.2	2.4
Fat	tioxazafen	0.010	7	99.6	4.4	4.4
		0.10	3	107.3	1.2	1.1
		0.50	3	98.7	1.5	1.5
	Benzamidine	0.010	7	71.9	0.9	1.3
		0.10	3	96.7	1.5	1.6
	Benzonitrile	0.025	7	90.1	3.4	3.8
		0.25	3	94.7	0.6	0.6
Liver	tioxazafen	0.010	8	92.8	3.3	3.6
		0.10	4	99.0	3.7	3.8
	Benzamidine	0.010	9	76.1	1.5	2.0
		0.10	5	94.0	5.9	6.2
		1.0	3	95.0	0.0	0.0
Muscle	tioxazafen	0.010	7	97.1	4.1	4.2
		0.10	3	102.0	1.0	1.0
	Benzamidine	0.010	8	78.4	2.2	2.8
		0.10	4	94.8	1.9	2.0

[a] From hen feeding study (Brungardt, 2014)

[b] Fortifications were performed with tioxazafen, benzamidine and benzonitrile reference standard solutions.

[c] Individual recoveries met a 70-120% recovery acceptance criterion.

The results of the independent laboratory validation for tioxazafen and benzamidine in four representative animal matrices are summarised in Table 70.

Table 70 Independent Laboratory Validation Recovery Results for tioxazafen and Benzamidine

Matrix	Fortification Level (mg/kg)	N	tioxazafen			Benzamidine		
			Mean Recovery* (%)	SD	RSD (%)	Average Recovery* (%)	SD	RSD (%)
Bovine Kidney	0.01 0.1	5 5 Total =10	98 100	7 1	8 1 Mean = 5	96 100	9 11	9 11 Mean = 10
Bovine Muscle	0.01 0.1	5 5 Total =10	96 96	6 1	6 1 Mean = 4	89 92	2 1	2 1 Mean = 2
Bovine Milk	0.01 0.1	5 5 Total =10	94 100	11 2	12 2 Mean = 7	92 92	4 7	5 7 Mean = 6
Poultry Egg	0.01 0.1	5 5 Total =10	100 99	7 2	7 2 Mean = 5	88 94	4 5	4 5 Mean = 5

*Individual recoveries met the 70-120% acceptance criterion.

Radiovalidation of the extraction procedures of the method was conducted using goat and hen samples containing endogenous radiolabelled residues, which were obtained from the respective metabolism studies of tioxazafen in lactating goats and laying hens. The results of the radiovalidation are summarised in Table 71. The radiovalidation experiments demonstrated generally comparable recoveries of tioxazafen, benzamidine, 2-thenoylglycine and benzonitrile from animal matrices using the analytical method extraction procedures compared to the metabolism study extraction procedures. Therefore, the method is acceptable for use in the determination of residues of tioxazafen and its metabolites benzamidine, 2-thenoylglycine and benzonitrile in animal matrices.

Table 71 Comparison of Recovery Results in Endogenous Method Validation and the Livestock Metabolism Studies

Analyte and Matrix	Extraction Method	Extraction Solvent	Extract -ability (%)	Analyte Percent TRR	Analyte mg/kg (mg/kg)	Analyte Relative Extraction Efficiency (%)**
tioxazafen						
Hen Egg	Metabolism	ACN/water; ACN	22.6	3.1	0.004	100
	Residue	80:20 ACN/H_2O	26.6	3.1	0.004	
Hen Subcutaneous Fat	Metabolism	Hexane/acetone; acetone	91.8	18.7	0.008	128
	Residue	1:1 ACN/hexane	84.1	23.9	0.008	
Benzonitrile						
Hen Subcutaneous Fat	Metabolism	Hexane/acetone; acetone	91.8	30.1	0.013	73.8
	Residue	1:1 ACN/hexane	84.1	22.2	0.008	
Benzamidine						
Goat Loin Muscle	Metabolism	ACN/water; ACN	99.5	98.9	0.054	82.8
	Residue	80:20 ACN/H_2O	84.5	81.9	0.044	
Goat Omental Fat	Metabolism	Hexane/acetone; acetone	63.8	25.6	0.004	169
	Residue	1:1 ACN/hexane	80.0	43.2	0.006	
Hen Egg	Metabolism	ACN/water; ACN	22.6	2.8	0.004	111
	Residue	80:20 ACN/H_2O	26.6	3.1	0.004	
2-Thenoylglycine						
Whole Milk	Metabolism*	NA	88.1	51.5	0.052	116
	Residue	Acetonitrile	75.1	59.5	0.059	
Goat Kidney	Metabolism	ACN/water; ACN	35.6	21.5	0.047	94.0
	Residue	80:20 ACN/H_2O	30.7	20.2	0.045	

*Skim milk and milk fat were analysed in the metabolism study, but whole milk was analysed in the residue study; therefore, the extractability and residues reported for whole milk were calculated based on the TRR of skim milk and milk fat.

**Analyte Relative Extraction Efficiency (%) is the Analyte Percent TRR for the residue extraction procedure divided by the Analyte Percent TRR for the metabolism study extraction procedure expressed as a percentage.

Stability of residues in stored analytical sample

Plant matrices

The storage stability of tioxazafen and benzamidine in representative crop raw agricultural commodities during frozen storage was determined for a period of nine months (Urbanczyk-Wochniak and Riter, 2014 MSL0025805). Stability samples for all crops were prepared by fortifying homogenized bulk samples of crop matrices separately with tioxazafen and benzamidine each at a concentration of 0.1 mg/kg (benzamidine as tioxazafen equivalents). Maize grain, lettuce leaves, radish root and whole orange fruit samples were removed from frozen storage and analysed at intervals of 0 and 1 days, and 1, 3, 6 and 9 months. Soya bean seed and lentil seed samples were removed from frozen storage and analysed at intervals of 0 and 1 days, and 1, 3, 5, 6 and 9 months. Fresh fortification recovery samples for all crops were fortified at each time point at the following concentrations: 0.01 mg/kg and 0.1 mg/kg. Analysis was conducted for tioxazafen by electron impact (EI) GC-MS/MS (ME-1604) and for benzamidine by LC-MS/MS with electrospray ionization (ESI) (ME-1579).

The recovery results from the analyses of the individual stored samples of each RAC are summarised in table 72. All benzamidine residues are reported in tioxazafen equivalents. All untreated control samples contained residues of tioxazafen and benzamidine below 0.0025 mg/kg (mg/kg).

Table 72 Remaining of tioxazafen and Benzamidine in RAC Stored at < -20 °C

RAC	Sampling time	tioxazafen (0.1 mg/kg fortification)		Sampling Time	Benzamidine (0.1 mg/kg fortification)	
		Remaining	procedural Recovery %		Remaining	Procedural Recovery%
Maize Grain	Day 0	0.0823	93.8	Day 0	0.105	107
	Day 1	0.092	101	Day 1	0.111	109
	Month 1 (Day 35)	0.0914	97.7	Month 1 (Day 35)	0.117	112
	Month 3 (Day 91)	0.0909	103	Month 3 (Day 90)	0.109	106
	Month 6 (Day 185)	0.0963	101	Month 6 (Day 188)	0.111	102
	Month 9 (Day 279)	0.0907	97.2	Month 9 (Day 273)	0.103	105
Lettuce Leaves	Day 0	0.0884	94	Day 0	0.0995	114
	Day 1	0.0884	105	Day 1	0.114	115
	Month 1 (Day 35)	0.0932	101	Month 1 (Day 35)	0.114	112
	Month 3 (Day 91)	0.0931	99	Month 3 (Day 90)	0.112	106
	Month 6 (Day 185)	0.0927	100	Month 6 (Day 188)	0.11	105
	Month 9* (Day 279)	0.0877	96	Month 9 (Day 273)	0.114	108
Radish Root	Day 0	0.102	100	Day 0	0.105	116
	Day 1	0.0961	103	Day 1	0.103	113
	Month 1 (Day 33)	0.1	97	Month 1 (Day 33)	0.112	108
	Month 3 (Day 89)	0.959	99	Month 3 (Day 88)	0.112	105
	Month 6 (Day 183)	0.101	101	Month 6 (Day 186)	0.112	102
	Month 9 (Day 277)	0.985	100	Month 9 (Day 271)	0.111	105
Whole Orange Fruit	Day 0	0.0915	92	Day 0	0.1	115
	Day 1	0.0894	95	Day 1	0.103	114
	Month 1 (Day 33)	0.0865	98	Month 1 (Day 33)	0.116	111
	Month 3 (Day 89)	0.092	100	Month 3 (Day 88)	0.109	105
	Month 6 (Day 183)	0.0867	99	Month 6 (Day 186)	0.108	104
	Month 9	0.0857	99	Month 9	0.114	107

RAC	Sampling time	tioxazafen (0.1 mg/kg fortification)		Sampling Time	Benzamidine (0.1 mg/kg fortification)	
		Remaining	procedural Recovery %		Remaining	Procedural Recovery%
	(Day 277)			(Day 271)		
Soya bean Seed	Day 0	0.0817	93	Day 0	0.109	108
	Day 1	0.107	103	Day 1	0.0972	105
	Month 1 (Day 33)	0.0926	94	Month 1 (Day 33)	0.114	113
	Month 3 (Day 123)	0.0956	100	Month 3 (Day 102)	0.0821	104
	Month 5 (Day 161)	0.0917	98	Month 5 (Day 160)	0.0713	97
	Month 6 (Day 193)	0.0901	103	Month 6 (Day 187)	0.0806	98
	Month 9 (Day 295)	0.0959	98	Month 9 (Day 295)	0.106	103
Lentil Seed	Day 0	0.0873	92	Day 0	0.107	108
	Day 1	0.1	101	Day 1	0.107	105
	Month 1 (Day 33)	0.0877	94	Month 1 (Day 33)	0.116	113
	Month 3 (Day 99)	0.104	106	Month 3 (Day 92)	0.0952	104
	Month 5 (Day 161)	0.0946	99	Month 5 (Day 160)	0.088	97
	Month 6 (Day 193)	0.101	101	Month 6 (Day 187)	0.0828	98
	Month 9 (Day 295)	0.0969	95	Month 9 (Day 295)	0.108	103

* REG20130010-00262 (lettuce sample analysed at 9 month time point) was identified as an outlier during statistical analysis.

In summary, the residues of tioxazafen and benzamidine in high water content (lettuce and radish), high acid content (orange), high oil content (soya bean), high protein content (lentil) and high starch conten (maize) matrix stored at < -20 °C is stable for at least 9 month

Animal Commodities

The stability of tioxazafen and its metabolites benzamidine, 2-thenoylglycine and benzonitrile in representative animal matrices during frozen storage was assessed (Brungardt, 2014, MSL0026368). The matrices selected for evaluation included milk, kidney and fat from cattle, and liver, muscle and eggs from poultry. The stability of tioxazafen and benzamidine was evaluated in all selected matrices. In addition, the stability of 2-thenoylglycine was evaluated in milk, liver and kidney, and the stability of benzonitrile was evaluated in fat.

Storage conditions and duration were chosen to represent those utilised for storage of residue samples during the poultry and cattle feeding studies. Samples were prepared by fortifying homogenized control matrices with the analytes of interest for that matrix at 10× the method LOQ (either 0.1 or 0.25 mg/kg). Samples were removed from frozen storage and analysed at nominal intervals of two and four months (two and three months for egg and fat). Control and fresh fortification recovery samples (at 1× and 10× the LOQ) were analysed at each of these time points as well as at Day 0. Tioxazafen and benzonitrile were analysed by EI GC-MS/MS, and benzamidine and 2-thenoylglycine by ESI LC-MS/MS using analytical method AG-ME-1764.

All analytes in the stored animal matrix samples were considered stable (degradation <30%) under frozen storage conditions for a period of at least six months.

The recovery results from the analyses of the individual stored animal matrices samples are summarised in Table 73. The recovery levels reported in these tables are not corrected for the background level in the corresponding control samples or the moisture content of the samples.

Table 73 Summary of Tioxazafen, Benzamidine and 2-Thenoylglycine Recoveries from Fresh Fortified Milk Samples

Time Point Months[a] (days)	Fort. Level	Number of Fortified Samples	tioxazafen Low = 0.010 mg/kg, High = 0.100 mg/kg		Benzamidine Low = 0.010 mg/kg, High = 0.100 mg/kg		2-Thenoylglycine Low = 0.010 mg/kg, High = 0.100 mg/kg	
			Remaining[b]	%RSD	Remaining[b]	%RSD	Remaining[b]	%RSD
Month 0	Low	5	1.03	4.69	1.04	1.80	1.10	4.81
(Day 0)	High[c]	5	0.97	0.47	1.04	0.86	0.983	1.94
Month 2	Low	3	1.01	11.4	0.911	4.77	0.99	2.67
(Day 63)	High	3	0.987	1.75	0.886	10.6	0.97	0.96
Month 4	Low	3	0.993	2.32	0.741	0.54	0.982	2.85
(Day 134)	High	3	1.13	0.64	0.976	1.69	1.01	1.63
Month 7	Low	3	1.06	10	0.903	1.8	0.985	0.2
(Day 225)	High	3	1.01	1.9	1.09	0.0	0.912	0.6

[a] Nominal month as stated in the protocol and amendments.

[b] Recovery values were not corrected for background levels in controls. Benzamidine and 2-thenoylglycine were fortified and measured in tioxazafen equivalents.

[c] The high Day 0 fresh fortification samples were considered to be the 0-day stability samples.

Average percent recoveries of tioxazafen, benzamidine and 2-thenoylglycine in fresh fortified liver samples are summarised in Table 74 below.

Table 74 Summary of tioxazafen, Benzamidine and 2-Thenoylglycine Recoveries from Fresh Fortified Liver Samples

Time Point Months[a] (days)	Fort. Level	Number of Fortified Samples	tioxazafen Low = 0.010 mg/kg, High = 0.100 mg/kg		Benzamidine Low = 0.010 mg/kg, High = 0.100 mg/kg		2-Thenoylglycine Low = 0.025 mg/kg, High = 0.250 mg/kg	
			Remaining[b]	%RSD	Remaining[b]	%RSD	Remaining[b]	%RSD
Month 0	Low	5	1.04	4.26	0.898	1.35	1.05	2.73
(Day 0)	High[c]	5	1.00	1.02	0.784	0.72	0.978	0.94
Month 2	Low	3	0.91	3.30	0.944	1.18	1.07	4.10
(Day 57)	High	3	1.02	1.43	0.916	0.82	1.05	2.41
Month 4	Low	3	97.3	1.19	0.693	1.96	0.897	3.46
(Day 120)	High	3	1.08	1.22	0.958	0.84	0.945	0.65
Month 7	Low	3	0.947	4.4	0.856	2.0	1.06	3.7
(Day 208)	High	3	0.925	2.1	0.954	0.52	0.887	1.8

[a] Nominal month as stated in the protocol and amendments.

[b] Recovery values were not corrected for background levels in controls. Benzamidine and 2-thenoylglycine were fortified and measured in tioxazafen equivalents.

[c] The high Day 0 fresh fortification samples were considered to be the 0-day stability samples.

Average percent recoveries of tioxazafen, benzamidine and 2-thenoylglycine in fresh fortified kidney samples are summarised in Table 75 below.

Table 75 Summary of tioxazafen, Benzamidine and 2-Thenoylglycine Recoveries from Fresh Fortified Kidney Samples

Time Point Months[a] (days)	Fort. Level	Number of Fortified Samples	tioxazafen Low = 0.010 mg/kg, High = 0.100 mg/kg		Benzamidine Low = 0.010 mg/kg, High = 0.100 mg/kg		2-Thenoylglycine Low = 0.025 mg/kg, High = 0.250 mg/kg	
			Remaining[b]	%RSD	Remaining[b]	%RSD	Remaining[b]	%RSD
Month 0	Low	5	1.01	6.19	0.939	1.00	1.10	2.53
(Day 0)	High[c]	5	0.985	0.73	0.816	1.61	0.998	0.36
Month 2	Low	3	1.01	10.7	0.924	1.19	0.971	4.74
(Day 58)	High	3	1.03	1.87	0.92	0.98	1.06	0.38
Month 4	Low	3	0.917	1.26	0.676	1.43	0.909	5.25
(Day 120)	High	3	1.14	1.02	0.951	0.11	0.951	0.49
Month 7	Low	3	0.927	0.6	0.85	0.4	1.20	5.3
(Day 209)	High	3	0.925	1.4	0.978	0.7	0.913	0.7

[a] Nominal month as stated in the protocol and amendments.

[b] Recovery values were not corrected for background levels in controls. Benzamidine and 2-thenoylglycine were fortified and measured in tioxazafen equivalents.

[c] The high Day 0 fresh fortification samples were considered to be the 0-day stability interval.

Average percent recoveries of tioxazafen and benzamidine in fresh fortified muscle samples are summarised in Table 76 below.

Table 76 Summary of tioxazafen and benzamidine recoveries from fresh fortified muscle samples

Time Point Months [a] (days)	Fort. Level	Number of Fortified Samples	tioxazafen Low = 0.010 mg/kg, High = 0.100 mg/kg		Benzamidine Low = 0.010 mg/kg, High = 0.100 mg/kg	
			Remaining [b]	%RSD	Remaining [b]	%RSD
Month 0 (Day 0)	Low	5	1.15	2.44	0.905	5.65
	High [c]	5	1.02	1.77	0.846	6.89
Month 2 (Day 61)	Low	3	1.20	2.50	0.884	3.93
	High	3	0.999	2.80	0.809	2.29
Month 4 (Day 133)	Low	3	0.87	1.15	0.717	3.52
	High	3	1.10	0.58	0.928	2.30
Month 7 (Day 223)	Low	3	0.979	0.59	0.859	0.47
	High	3	0.924	1.4	0.986	1.3

[a] Nominal month as stated in the protocol and amendments.

[b] Recovery values were not corrected for background levels in controls. Benzamidine was fortified and measured in tioxazafen equivalents.

[c] The high Day 0 fresh fortification samples were considered to be the 0-day stability samples.

Average percent recoveries of tioxazafen, benzamidine and benzonitrile in fresh fortified fat samples are summarised in Table77 below.

Table 77 Summary of tioxazafen, Benzamidine and Benzonitrile Recoveries from Fresh Fortified Fat Samples

Time Point Months[a] (days)	Fort. Level	Number of Fortified Samples	tioxazafen Low = 0.010 mg/kg, High = 0.100 mg/kg		Benzamidine Low = 0.010 mg/kg, High = 0.100 mg/kg		Benzonitrile Low = 0.025 mg/kg, High = 0.250 mg/kg	
			Remaining [b]	%RSD	Remaining [b]	%RSD	Remaining [b]	%RSD
Month 0	Low	5	0.976	4.44	1.07	6.10	1.08	3.46
(Day 0)	High [c]	5	0.976	1.80	0.992	3.79	0.932	2.06
Month 2	Low	3	1.00	4.58	0.793	2.21	1.00	5.27
(Day 46)	High	3	0.989	1.11	0.950	3.80	0.869	3.18
Month 3	Low	3	1.01	0.57	0.812	1.30	1.08	0.37
(Day 104)	High	3	0.978	0.83	1.05	2.21	0.935	0.50
Month 6	Low	3	1.01	2.3	0.902	1.9	1.11	2.2
(Day 185)	High	3	0.967	0.78	1.14	1.8	0.965	1.0

[a] Nominal month as stated in the protocol and amendments.

[b] Recovery values were not corrected for background levels in controls. Benzamidine and benzonitrile were fortified and measured in tioxazafen equivalents.

[c] The high Day 0 fresh fortification samples were considered to be the 0-day stability samples.

Average percent recoveries of tioxazafen and benzamidine in fresh fortified egg samples are summarised in Table 78 below.

Table 78 Summary of tioxazafen and Benzamidine Recoveries from Fresh Fortified Egg Samples

Time Point Months [a] (days)	Fort. Level	Number of Fortified Samples	tioxazafen Low = 0.010 mg/kg, High = 0.100 mg/kg		Benzamidine Low = 0.010 mg/kg, High = 0.100 mg/kg	
			Remaining [b]	%RSD	Remaining [b]	%RSD
Month 0	Low	3	1.05	5.04	0.913	1.51
(Day 0)	High [c]	5	1.02	2.29	0.858	4.19
Month 2	Low	3	0.937	4.04	0.788	1.50
(Day 48)	High	3	1.1	1.84	0.95	0.64
Month 3	Low	3	0.87	4.14	0.908	0.61
(Day 111) [d]	High	3	0.943	1.70	1.05	0.95
Month 6	Low	3	0.895	1.70	0.872	2.30
(Day 187)	High	3	0.97	1.10	1.00	0.86

[a] Nominal month as stated in the protocol and amendments.

[b] Recovery values were not corrected for background levels in controls. Benzamidine was fortified and measured in tioxazafen equivalents.

[c] The high Day 0 fresh fortification samples were considered to be the 0-day stability samples.

[d] Day 110 for benzamidine

* Two additional QC samples were fortified at the LOQ; these samples appeared to be contaminated; data are not being reported.

Average results for the stored and fresh fortifications for each analyte at each time point for milk, liver, and kidney are summarised in Table 79 below. Fortification levels were either 0.1 or 1.25 mg/kg depending on matrix/analyte combination.

Table 79 Comparison of Recoveries of Stored and Freshly Fortified Milk, Liver, and Kidney Samples

Matrix	Actual Sampling Time	Tioxazafen [a]		Benzamidine [b]		2-Thenoylglycine [c]	
		Stored Samples [d] (Remaining)	Fresh Fortifications (Remaining)	Stored Samples [d] (Remaining)	Fresh Fortifications (Remaining)	Stored Samples [d] (Remaining)	Fresh Fortifications (Remaining)
Milk	Day 0	0.97	0.97	1.04	1.04	0.983	0.983
	Day 63	0.927	0.987	0.996	0.886	0.988	0.97
	Day 134	0.934	1.13	1.02	0.976	0.984	1.01
	Day 225	0.917	1.01	1.07	1.09	0.915	0.912
Liver	Day 0	1.00	1.00	0.784	0.784	0.978	0.978
	Day 57	1.02	1.02	0.853	0.916	1.06	1.05
	Day 120	0.893	1.08	0.781	0.958	0.982	0.945
	Day 208	0.917	0.925	0.837	0.954	0.91	0.887
Kidney	Day 0	0.985	0.985	0.816	0.816	0.998	0.998
	Day 58	1.02	1.03	0.886	0.92	1.16	1.06
	Day 120	0.923	1.14	0.795	0.951	1.03	0.951
	Day 209	0.914	0.925	0.845	0.978	1.02	0.913

[a] Milk, liver and kidney fortified with tioxazafen at 0.1 mg/kg

[b] Milk, liver and kidney fortified with benzamidine at 0.1 mg/kg (tioxazafen equivalents)

[c] Milk fortified with 2-thenoylglycine at 0.1 mg/kg; liver and kidney fortified at 0.25 mg/kg (tioxazafen equivalents)

[d] The high Day 0 Fresh Fortification samples were considered to be the Day 0 Stored Samples

Average results for the stored and fresh fortifications for each analyte at each time point for muscle and egg are summarised in Table 80 below. All fortification levels were at 0.1 mg/kg.

Table 80 Comparison of Recoveries of Stored and Freshly Fortified Muscle and Egg Samples

Matrix	Actual Sampling Time	Tioxazafen [a]		Benzamidine [b]	
		Stored Samples [d] (Remaining)	Fresh Fortifications (Remaining)	Stored Samples [d] (Remaining)	Fresh Fortifications (Remaining)
Muscle	Day 0	1.02	1.02	0.846	0.846
	Day 61	0.997	0.999	0.818	0.809

Matrix	Actual Sampling Time	Tioxazafen [a]		Benzamidine [b]	
		Stored Samples [d] (Remaining)	Fresh Fortifications (Remaining)	Stored Samples [d] (Remaining)	Fresh Fortifications (Remaining)
	Day 133	0.916	1.10	0.855	0.928
	Day 223	0.90	0.924	0.936	0.986
Egg	Day 0	1.02	1.02	0.858	0.858
	Day 48	0.98	1.01	1.02	0.95
	Day 111[c]	0.937	0.943	1.02	1.05
	Day 187	0.919	0.97	1.01	1.00

[a] Muscle and egg fortified with tioxazafen at 0.1 mg/kg.

[b] Muscle and egg fortified with benzamidine at 0.1 mg/kg (tioxazafen equivalents)

[c] Day 110 for benzamidine.

[d] The high Day 0 Fresh Fortification samples were considered to be the Day 0 Stored Samples

Average results for the 0.10 mg/kg stored and fresh fortifications for each analyte at each time point for fat are summarised in Table 81 below.

Table 81 Comparison of Recoveries of Stored and Freshly Fortified Fat Samples

Matrix	Actual Sampling Time	Tioxazafen [a]		Benzamidine [b]		Benzonitrile [c]	
		Stored Samples [d] (Remaining)	Fresh Fortifications (Remaining)	Stored Samples [d] (Remaining)	Fresh Fortifications (Remaining)	Stored Samples [d] (Remaining)	Fresh Fortifications (Remaining)
Fat	Day 0	0.976	0.976	0.992	0.992	0.932	0.932
	Day 46	0.941	0.989	1.02	0.95	0.856	0.869
	Day 104	0.993	0.978	1.07	1.05	0.932	0.935
	Day 185	0.952	0.967	1.05	1.14	0.938	0.965

[a] Fat fortified with tioxazafen at 0.1 mg/kg.

[b] Fat fortified with benzamidine at 0.1 mg/kg (tioxazafen equivalents)

[c] Fat fortified with benzonitrile at 0.25 mg/kg (tioxazafen equivalents)

[d] The high Day 0 Fresh Fortification samples were considered to be the Day 0 Stored Samples

The percent change of tioxazafen, benzamidine, 2-thenoylglycine, and benzonitrile samples stored frozen are summarised in Table 82.

The estimated degradation of individual analytes in animal matrices was calculated using a statistical regression model. The statistical analysis of the concentration change was evaluated over a period of 225 days for all matrices and analytes, although the period of storage ranged from 185-225 days. The results from the statistical analysis are summarised in Table 83. For four analytes in six animal commodity samples, only one statistically significant change in concentration was detected out of sixteen statistical tests at the 0.05 significance level, which was for tioxazafen in egg with an estimated degradation of 12.25% after 225 days of storage. The estimated concentration change after 225 days of storage under frozen conditions ranged from −13% to +16% across all analytes and matrices. Thus all analytes in the animal matrix samples were considered stable under frozen storage conditions for a period of at least six months by the criterion specified in the study protocol (i.e. analyte residues are considered stable unless degradation is >30%).

Table 82 Estimated Percent Change of tioxazafen and Benzamidine Concentrations in Animal Matrices over a Nominal Frozen Storage Period of Seven Months (225 Days)

Matrix	tioxazafen					Benzamidine				
	Initial		After 225 Days		Concentration Change (%)[a]	Initial		After 225 Days		Concentration Change (%)[a]
	Mean (mg/kg)	SE	Mean (mg/kg)	SE		Mean (mg/kg)	SE	Mean (mg/kg)	SE	
Egg	0.101	0.001	0.089	0.002	−12[c]	0.092	0.006	0.106	0.008	+16[b]
Fat	0.097	0.002	0.096	0.003	−0.9[b]	0.100	0.002	0.108	0.003	+7.9[b]
Kidney	0.100	0.003	0.090	0.004	−10[b]	0.084	0.004	0.084	0.005	+0.1[b]
Liver	0.101	0.004	0.089	0.005	−12[b]	0.080	0.003	0.083	0.004	+3.7[b]
Milk	0.096	0.001	0.091	0.001	−4.7[b]	0.102	0.003	0.105	0.003	+2.8[b]

Matrix	tioxazafen					Benzamidine				
	Initial		After 225 Days		Concentration Change (%)[a]	Initial		After 225 Days		Concentration Change (%)[a]
	Mean (mg/kg)	SE	Mean (mg/kg)	SE		Mean (mg/kg)	SE	Mean (mg/kg)	SE	
Muscle	0.102	0.002	0.089	0.002	−13[b]	0.082	0.003	0.092	0.003	+12[b]

[a] A positive number reflects an increase and a negative number reflects a decrease in concentration.

[b] Not a statistically significant change ($p > 0.05$).

[c] Statistically significant at the 0.05 level.

Table 83 Estimated Percent Change of 2-Thenoylglycine and Benzonitrile Concentrations in Animal Matrices over a Nominal Frozen Storage Period of Seven Months (225 Days)

Matrix	2-Thenoylglycine					Benzonitrile				
	Initial		After 225 Days		Concentration Change (%)[a]	Initial		After 225 Days		Concentration Change (%)[a]
	Mean (mg/kg)	SE	Mean (mg/kg)	SE		Mean (mg/kg)	SE	Mean (mg/kg)	SE	
Fat	na	na	na	na	na	0.225	0.009	0.235	0.013	+4.6[b]
Kidney	0.266	0.018	0.258	0.021	−3.0[b]	na	na	na	na	na
Liver	0.256	0.011	0.231	0.013	−9.8[b]	na	na	na	na	na
Milk	0.100	0.002	0.093	0.002	−6.7[b]	na	na	na	na	na

na Not applicable

[a] A positive number reflects an increase and a negative number reflects a decrease in concentration.

[b] Not a statistically significant change ($p > 0.05$).

Using a statistical regression model, the estimated change in concentration of MON 102100, benzamidine, 2-thenoylglycine and benzonitrile in animal matrices stored under frozen conditions for a period of 6-7 months ranged from −13 to +16%. These analytes are therefore considered stable in animal matrices in frozen storage (-18 °C)for a period of at least six months.

USE PATTERN

Tioxazafen has been registered in maize and soya bean in Canada and in maize, soya bean and cotton in the United States of America, to control nematodes. The information available to the Meeting on registered uses is summarised in Table 84.

Table 84 Registered uses of tioxazafen

Crop	Country	Formulation		Application			Remarks
		g ai/L	type	Method	Rate (g ai/ha)	mg ai/seed	
Pulse (Group 015)							
Soya bean	USA	541	SC	Seed treatment	154-309	0.25-0.5	Based on seeding rate of 625,000 seeds/ha; yearly max. is 0.314 kg ai/ha
Soya bean	Canada	537	SC	Seed treatment	125	0.25	Based on seeding rate of 500,000 seeds/ha
Cereal grains (Group 020)							
Maize	USA	541	SC	Seed treatment	50-99	0.5-1.0	Based on seeding rate of 100,625 seeds/ha; yearly max. is 0.10 kg ai/ha
Maize	Canada	537	SC	Seed treatment	44.5	0.5	Based on seeding rate of 89,000 seeds/ha
Oilseed (Group 023)							
Cotton	USA	541	SC	Seed treatment	105-210	0.5-1.0	Based on seeding rate of 212500 seeds/ha; yearly max. is 0.213 kg ai/ha

RESIDUES RESULTING FROM SUPERVISED TRIALS

Supervised trials have been conducted to support MRLs for tioxazafen used as seed treatment of maize, soya bean and cotton.

Maize (Group 020 Cereal Grain)

A study containing 22 field trials was conducted on maize following seed treatment with 48.7% or 45.8% (w/w) tioxazafen SC at rates of 0.5, 1.0 and 2.0 mg/seed (Mueth 2014 MSL0025349). A commercial fungicide and insecticide seed treatment (Acceleron®) was added to all treatments. Forage was collected at the late dough stage. Stover and grain were collected at maturity. For each commodity, two composite samples were collected from each treated plot and mean residue of two samples was selected. Samples were stored frozen after collection and were extracted for analysis within 186 days of sampling. All samples were analysed within 2 days after extraction with method AG-ME-1604 for the analyses of tioxazafen and AG- ME-1579 for benzamidine. The LOQs of methods were validated to 0.0025 mg/kg for both tioxazafen and benzamidine in maize RACs and processed fractions. Forage, grain and stover were analysed for tioxazafen and benzamidine. No residues of either tioxazafen or benzamidine above the LOQ (0.0025 mg/kg for each analyte) were detected in grain from any treatment

Table 85 Residues of tioxazafen and benzamidine in maize grain from supervised field trials in the USA after seed treatment with tioxazafen 48.7% w/w SC

Trial code, Location NAFTA Region Country, Years (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg			DAP [b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total [a]		
01PA Lehigh Pennsylvania Region 1 USA, 2012 (DKC53-78)	0.53	0.039	Grain	<0.0025	<0.0025	<0.0050	129	Trt. 2 MSL0025349
	1.02	0.076	Grain	<0.0025	<0.0025	<0.0050	129	Trt. 3 MSL0025349
	2.1	0.156	Grain	<0.0025	<0.0025	<0.0050	129	Trt. 4 MSL0025349
02NC Wayne North Carolina Region 2 USA, 2012 (DKC58-83)	0.53	0.032	Grain	<0.0025	<0.0025	<0.0050	134	Trt. 2 MSL0025349
	1.04	0.068	Grain	<0.0025	<0.0025	<0.0050	134	Trt. 3 MSL0025349
	2.14	0.132	Grain	<0.0025	<0.0025	<0.0050	134	Trt. 4 MSL0025349
03MI Ottawa Michigan Region 5 USA,2012 (DKC46-07)	0.51	0.036	Grain	<0.0025	<0.0025	<0.0050	138	Trt. 2 MSL0025349
	1.03	0.072	Grain	<0.0025	<0.0025	<0.0050	138	Trt. 3 MSL0025349
	2.05	0.143	Grain	<0.0025	<0.0025	<0.0050	138	Trt. 4 MSL0025349
04IN Elkhart Indiana Region 5 USA,2012 (DKC53-78)	0.53	0.037	Grain	<0.0025	<0.0025	<0.0050	149	Trt. 2 MSL0025349
	1.02	0.071	Grain	<0.0025	<0.0025	<0.0050	149	Trt. 3 MSL0025349
	2.1	0.151	Grain	<0.0025	<0.0025	<0.0050	149	Trt. 4 MSL0025349
05IL Clinton Illinois Region 5 USA, 2012 (DKC62-97)	0.54	0.039	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 2 MSL0025349
	1.07	0.077	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 3 MSL0025349
	2.26	0.164	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 4 MSL0025349
06IL Stark Illinois Region 5 USA, 2012 (DKC62-97)	0.54	0.044	Grain	<0.0025	<0.0025	<0.0050	144	Trt. 2 MSL0025349
	1.07	0.087	Grain	<0.0025	<0.0025	<0.0050	144	Trt. 3 MSL0025349
	2.26	0.184	Grain	<0.0025	<0.0025	<0.0050	144	Trt. 4 MSL0025349
07IL Carroll Illinois Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 2 MSL0025349
	1.04	0.079	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 3 MSL0025349
	2.14	0.162	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 4 MSL0025349
08IL Ogle Illinois Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 2 MSL0025349
	1.04	0.079	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 3 MSL0025349
	2.14	0.162	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 4 MSL0025349
09IA Wapello Iowa Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Grain	<0.0025	<0.0025	<0.0050	146	Trt. 2 MSL0025349
	1.04	0.079	Grain	<0.0025	<0.0025	<0.0050	146	Trt. 3 MSL0025349
	2.14	0.163	Grain	<0.0025	<0.0025	<0.0050	146	Trt. 4 MSL0025349
10IA Jefferson Iowa Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Grain	<0.0025	<0.0025	<0.0050	153	Trt. 2 MSL0025349
	1.04	0.079	Grain	<0.0025	<0.0025	<0.0050	153	Trt. 3 MSL0025349
	2.14	0.162	Grain	<0.0025	<0.0025	<0.0050	153	Trt. 4 MSL0025349
11IA Clinton Iowa Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 2 MSL0025349
	1.04	0.079	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 3 MSL0025349
	2.14	0.162	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 4 MSL0025349
12IA Louisa Iowa Region 5 USA, 2012	0.53	0.04	Grain	<0.0025	<0.0025	<0.0050	165	Trt. 2 MSL0025349
	1.04	0.079	Grain	<0.0025	<0.0025	<0.0050	165	Trt. 3 MSL0025349

Trial code, Location NAFTA Region Country, Years (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg			DAP [b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total [a]		
(DKC58-83	2.14	0.162	Grain	<0.0025	<0.0025	<0.0050	165	Trt. 4 MSL0025349
13IA Greene Iowa Region 5 USA, 2012(DKC59-35)	0.53	0.036	Grain	<0.0025	<0.0025	<0.0050	126	Trt. 2 MSL0025349
	1.06	0.073	Grain	<0.0025	<0.0025	<0.0050	126	Trt. 3 MSL0025349
	2.15	0.148	Grain	<0.0025	<0.0025	<0.0050	126	Trt. 4 MSL0025349
14MO Adair Missouri Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Grain	<0.0025	<0.0025	<0.0050	128	Trt. 2 MSL0025349
	1.04	0.079	Grain	<0.0025	<0.0025	<0.0050	128	Trt. 3 MSL0025349
	2.14	0.162	Grain	<0.0025	<0.0025	<0.0050	128	Trt. 4 MSL0025349
15NE York Nebraska Region 5 USA, 2012 (DKC59-35)	0.53	0.042	Grain	<0.0025	<0.0025	<0.0050	152	Trt. 2 MSL0025349
	1.06	0.087	Grain	<0.0025	<0.0025	<0.0050	152	Trt. 3 MSL0025349
	2.15	0.181	Grain	<0.0025	<0.0025	<0.0050	152	Trt. 4 MSL0025349
16NE Polk Nebraska Region 5 USA, 2012 (DKC59-35)	0.53	0.035	Grain	<0.0025	<0.0025	<0.0050	152	Trt. 2 MSL0025349
	1.06	0.07	Grain	<0.0025	<0.0025	<0.0050	152	Trt. 3 MSL0025349
	2.15	0.145	Grain	<0.0025	<0.0025	<0.0050	152	Trt. 4 MSL0025349
17NE Fillmore Nebraska Region 5 USA, 2012 (DKC59-35)	0.53	0.041	Grain	<0.0025	<0.0025	<0.0050	134	Trt. 2 MSL0025349
	1.06	0.081	Grain	<0.0025	<0.0025	<0.0050	134	Trt. 3 MSL0025349
	2.15	0.169	Grain	<0.0025	<0.0025	<0.0050	134	Trt. 4 MSL0025349
18MN Freeborn Minnesota Region 5 USA, 2012 (DKC46-07)	0.51	0.044	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 2 MSL0025349
	1.03	0.089	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 3 MSL0025349
	2.05	0.177	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 4 MSL0025349
19MN Steele Minnesota Region 5 USA, 2012 (DKC46-07)	0.51	0.044	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 2 MSL0025349
	1.03	0.089	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 3 MSL0025349
	2.05	0.177	Grain	<0.0025	<0.0025	<0.0050	154	Trt. 4 MSL0025349
20WI Walworth Wisconsin Region 5 USA, 2012 (DKC53-78)	0.53	0.039	Grain	<0.0025	<0.0025	<0.0050	150	Trt. 2 MSL0025349
	1.02	0.076	Grain	<0.0025	<0.0025	<0.0050	150	Trt. 3 MSL0025349
	2.1	0.155	Grain	<0.0025	<0.0025	<0.0050	150	Trt. 4 MSL0025349
21TX Tom Green Texas Region 6 USA, 2012 (DKC62-97)	0.54	0.042	Grain	<0.0025	<0.0025	<0.0050	118	Trt. 2 MSL0025349
	1.07	0.081	Grain	<0.0025	<0.0025	<0.0050	118	Trt. 3 MSL0025349
	2.26	0.173	Grain	<0.0025	<0.0025	<0.0050	118	Trt. 4 MSL0025349
22OK Caddo Oklahoma Region 6 USA, 2012 (DKC59-35)	0.53	0.046	Grain	<0.0025	<0.0025	<0.0050	113	Trt. 2 MSL0025349
	1.06	0.093	Grain	<0.0025	<0.0025	<0.0050	113	Trt. 3 MSL0025349
	2.15	0.189	Grain	<0.0025	<0.0025	<0.0050	113	Trt. 4 MSL0025349

[a] Total mg/kg = (Tioxazafen mg/kg) + (Benzamidine mg/kg). Benzamidine is reported in Tioxazafen equivalents. Values below the LOQ of 0.0025 mg/kg are summed as 0.0025.

[b] DAP = Interval in days between planting of treated seed and harvesting of commodity. DAP for individual replicates is listed after the average value.

Soya bean (Crop Group 015 Pulses)

A study containing 22 field trials was conducted on soya beans following seed treatment with 47.3% (w/w) tioxazafen SC at rates of 0.5 and 1.0 mg/seed (Mueth 2014 MSL0025350). Three treatments (one untreated control, maximum proposed seed treatment rate and an exaggerated rate of tioxazafen) were included in the residue trials. Soya bean forage, hay and seed commodities were collected at the normal harvest time. Forage was collected when the plants were ~7 days past the R1-R2 growth stage. Hay was collected when the plants were ~14 days past the R1-R2 growth stage and seed samples were collected at maturity. For each commodity, two composite samples were collected from each treated plot and mean residue of two samples was selected. All forage samples in this study were extracted and analysed within a period of less than 218 days after sampling. All seed samples were extracted within 133 days and all hay samples were extracted within 201 days after sampling, except one decline sample that was reanalysed for tioxazafen after 317 days and one control sample that was reanalysed after 305 days. All samples were analysed within two days after extraction with method AG-ME-1604 for the analyses of tioxazafen and AG- ME-1579 for benzamidine. The LOQs of methods were validated to 0.0025 mg/kg for both tioxazafen and benzamidine in soya bean RACs and processed fractions. Benzamidine levels are expressed in tioxazafen equivalents. Forage, hay and seed were analysed for tioxazafen and benzamidine.

Residues in seed totalled up to about 0.05 mg/kg, and were comprised primarily of benzamidine, with only trace levels of tioxazafen. The magnitude of tioxazafen and benzamidine residues in soya bean RACs are summarised below in Table 86.

Table 86 Residues of tioxazafen and benzamidine in soya bean from supervised field trials in the USA after seed treatment of tioxazafen SC

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP[b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total[a]		
01NC Wayne North Carolina Region 2 USA, 2013 (AG6132)	0.57	0.244	Seed	<0.0025	0.0126	0.0151	176	Trt. 2 MSL0025350
	0.93	0.38	Seed	<0.0025	0.0178	0.0203	176	Trt. 3 MSL0025350
02SC Barnwell South Carolina Region 2 USA, 2013 (AG6132)	0.57	0.163	Seed	<0.0025	0.0146	0.0171	131	Trt. 2 MSL0025350
	0.93	0.266	Seed	<0.0025	0.0114	0.0139	131	Trt. 3 MSL0025350
03AR Crittenden Arkansas Region 4 USA, 2013 (AG4832)	0.53	0.19	Seed	0.0038	0.0164	0.0202	131	Trt. 2 MSL0025350
	0.93	0.333	Seed	<0.0025	0.0223	0.0248	131	Trt. 3 MSL0025350
04MO Butler Missouri Region 4 USA, 2013 (AG4832)	0.53	0.183	Seed	<0.0025	0.0038	0.0063	153	Trt. 2 MSL0025350
	0.93	0.32	Seed	<0.0025	0.0204	0.0229	153	Trt. 3 MSL0025350
05MS Washington Mississippi Region 4 USA, 2013 (AG4832)	0.53	0.17	Seed	<0.0025	0.0155	0.018	132	Trt. 2 MSL0025350
	0.93	0.298	Seed	<0.0025	0.0291	0.0316	132	Trt. 3 MSL0025350
06LA Rapides Louisiana Region 4 USA, 2013 (AG4832)	0.53	0.257	Seed	<0.0025	0.0281	0.0306	128	Trt. 2 MSL0025350
	0.93	0.472	Seed	<0.0025	0.0449	0.0474	128	Trt. 3 MSL0025350
07MI Ottawa Michigan Region 5 USA, 2013 (AG2031)	0.54	0.173	Seed	<0.0025	0.0049	0.0074	145	Trt. 2 MSL0025350
	1.09	0.352	Seed	<0.0025	0.0069	0.0094	145	Trt. 3 MSL0025350
08IN Montgomery Indiana Region 5 USA, 2013 (AG4130)	0.54	0.222	Seed	<0.0025	0.0074	0.0099	156	Trt. 2 MSL0025350
	0.96	0.364	Seed	<0.0025	0.0157	0.0182	156	Trt. 3 MSL0025350
09IL Clinton Illinois Region 5 USA, 2013 (AG4130)	0.54	0.23	Seed	<0.0025	0.0103	0.0128	111	Trt. 2 MSL0025350
	0.96	0.355	Seed	<0.0025	0.0215	0.024	111	Trt. 3 MSL0025350
10IL Madison Illinois Region 5 USA, 2013 (AG4130)	0.54	0.208	Seed	<0.0025	0.013	0.0155	113	Trt. 2 MSL0025350
	0.96	0.37	Seed	<0.0025	0.032	0.0345	113	Trt. 3 MSL0025350
11IL Carroll Illinois Region 5 USA, 2013 (AG3130)	0.46	0.148	Seed	<0.0025	0.0071	0.0096	128	Trt. 2 MSL0025350
	0.88	0.284	Seed	<0.0025	0.0156	0.0181	128	Trt. 3 MSL0025350
12IA Jefferson Iowa Region 5 USA, 2013 (AG3130)	0.46	0.148	Seed	<0.0025	0.0069	0.0094	123	Trt. 2 MSL0025350
	0.88	0.192	Seed	<0.0025	0.0098	0.0123	123	Trt. 3 MSL0025350
13IA Wapello Iowa Region 5 USA, 2013 (AG3130)	0.46	0.148	Seed	<0.0025	0.0049	0.0074	120	Trt. 2 MSL0025350
	0.88	0.284	Seed	<0.0025	0.0074	0.0099	120	Trt. 3 MSL0025350

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP[b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total[a]		
14IA Louisa Iowa Region 5 USA, 2013 (AG3130)	0.46	0.148	Seed	<0.0025	0.0067	0.0092	134	Trt. 2 MSL0025350
	0.88	0.284	Seed	<0.0025	0.0136	0.0161	134	Trt. 3 MSL0025350
15IA Clinton Iowa Region 5 USA, 2013 (AG3130)	0.46	0.148	Seed	<0.0025	0.0055	0.008	128	Trt. 2 MSL0025350
	0.88	0.284	Seed	<0.0025	0.0076	0.0101	128	Trt. 3 MSL0025350
16MO Adair Missouri Region 5 USA, 2013 (AG3130)	0.46	0.148	Seed	<0.0025	0.0129	0.0154	135	Trt. 2 MSL0025350
	0.88	0.284	Seed	<0.0025	0.0174	0.0199	135	Trt. 3 MSL0025350
17NE York Nebraska Region 5 USA, 2013 (AG3130)	0.46	0.196	Seed	<0.0025	0.0094	0.0119	141	Trt. 2 MSL0025350
	0.88	0.385	Seed	<0.0025	0.0162	0.0187	141	Trt. 3 MSL0025350
18NE Polk Nebraska Region 5 USA, 2013 (AG3130)	0.46	0.163	Seed	<0.0025	0.0135	0.016	132	Trt. 2 MSL0025350
	0.88	0.322	Seed	<0.0025	0.0218	0.0243	132	Trt. 3 MSL0025350
19NE Seward Nebraska Region 5 USA, 2013 (AG3130)	0.46	0.16	Seed	<0.0025	0.0194	0.0219	120	Trt. 2 MSL0025350
	0.88	0.308	Seed	<0.0025	0.0222	0.0247	120	Trt. 3 MSL0025350
20MN Freeborn Minnesota Region 5 USA, 2013 (AG2031)	0.54	0.207	Seed	<0.0025	0.0037	0.0062	134	Trt. 2 MSL0025350
	1.09	0.42	Seed	<0.0025	0.006	0.0085	134	Trt. 3 MSL0025350
21MN Steele Minnesota Region 5 USA, 2013 (AG2031)	0.54	0.204	Seed	<0.0025	0.003	0.0055	136	Trt. 2 MSL0025350
	1.09	0.418	Seed	<0.0025	0.0049	0.0074	136	Trt. 3 MSL0025350
22WI Walworth Wisconsin Region 5 USA, 2013 (AG2031)	0.54	0.227	Seed	<0.0025	0.01	0.0125	127	Trt. 2 MSL0025350
	1.09	0.458	Seed	<0.0025	0.0086	0.0111	127	Trt. 3 MSL0025350

[a] Total mg/kg = (Tioxazafen mg/kg) + (Benzamidine mg/kg). Benzamidine is reported in Tioxazafen equivalents. Values below the LOQ of 0.0025 mg/kg are summed as 0.0025.

[b] DAP = Interval in days between planting of treated seed and harvesting of commodity. DAP for individual replicates is listed after the average value.

Cotton (Crop Group 023 Oilseed)

A study containing 13 field trials was conducted on cotton following seed treatment with 47.3 or47.8% (w/w) tioxazafen SC at rates of 1.0 and 2.0 mg/seed (Mueth 2014 MSL0025351). Three treatments (one untreated control, maximum proposed label rate and an exaggerated rate of tioxazafen) were included in the residue trials. At all sites, undelinted cotton seed commodities were collected at the normal harvest time. At four sites (08TX, 09TX, 10OK, and 11TX) cotton gin by-products were also collected at the normal harvest time. At two sites (08TX and 09TX) decline samples of cotton gin by-products and undelinted seed were collected from Treatment 3. In addition to the normal harvest samples, these samples were collected 7 days before normal harvest and at 7 and 14 days after normal harvest. For each commodity, a single composite sample was collected from each untreated control plot and two composite samples were collected from each treated plot, and mean residue of two samples was selected. All cotton gin by-

products samples in this study were extracted and analysed within a period of less than 127 days after sampling. All cotton seed samples were extracted within 190 days after sampling. All samples were analysed within 4 days after extraction with method AG-ME-1604 for the analyses of tioxazafen and AG- ME-1579 for benzamidine. The LOQs of methods were validated to 0.0025 mg/kg for both tioxazafen and benzamidine in cotton RACs. Benzamidine levels are expressed in tioxazafen equivalents. Undelinted cotton seed and Gin by-products were analysed for tioxazafen and benzamidine. The magnitude of tioxazafen and benzamidine residues in cotton RACs are summarised below in Table 87.

Table 87 Residues of tioxazafen and benzamidine in cotton from supervised field trials in USA after seed treatment of tioxazafen SC

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP[b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total[a]		
01SC Barnwell South Carolina Region 2 USA, 2013 (DP 1044)	0.96	0.138	Seed	<0.0025	<0.0025	<0.0050	178	Trt. 2 MSL0025351
	2.07	0.297	Seed	<0.0025	<0.0025	<0.0050	178	Trt. 3 MSL0025351
02GA Tift Georgia Region 2 USA, 2013 (DP 1044)	0.96	0.103	Seed	<0.0025	<0.0025	<0.0050	162	Trt. 2 MSL0025351
	2.07	0.223	Seed	<0.0025	<0.0025	<0.0050	162	Trt. 3 MSL0025351
03MS Washington Mississippi Region 4 USA, 2013 (DP 1044)	0.96	0.137	Seed	<0.0025	<0.0025	<0.0050	152	Trt. 2 MSL0025351
	2.07	0.294	Seed	<0.0025	<0.0025	<0.0050	152	Trt. 3 MSL0025351
04MO Butler Missouri Region 4 USA, 2013 (DP 1044)	0.96	0.124	Seed	<0.0025	<0.0025	<0.0050	181	Trt. 2 MSL0025351
	2.07	0.267	Seed	<0.0025	<0.0025	<0.0050	181	Trt. 3 MSL0025351
05LA Rapides Louisiana Region 4 USA, 2013 (DP 1044)	0.96	0.138	Seed	<0.0025	<0.0025	<0.0050	147	Trt. 2 MSL0025351
	2.07	0.268	Seed	<0.0025	<0.0025	<0.0050	147	Trt. 3 MSL0025351
06TX Uvalde Texas Region 6 USA, 2013 (DP 1044)	0.96	0.124	Seed	<0.0025	<0.0025	<0.0050	156	Trt. 2 MSL0025351
	2.07	0.267	Seed	<0.0025	<0.0025	<0.0050	156	Trt. 3 MSL0025351
07TX Willacy Texas Region 6 USA, 2013 (DP 1044)	0.96	0.132	Seed	<0.0025	<0.0025	<0.0050	146	Trt. 2 MSL0025351
	2.07	0.284	Seed	<0.0025	<0.0025	<0.0050	146	Trt. 3 MSL0025351
08TX Tom Green Texas Region 8 USA, 2013 (DP 1044)	0.96	0.11	Seed	<0.0025	<0.0025	<0.0050	151	Trt. 2 MSL0025351
	2.07	0.238	Seed	<0.0025	<0.0025	<0.0050	151	Trt. 3 MSL0025351
09TX	0.96	0.172	Seed	<0.0025	<0.0025	<0.0050	170	Trt. 2 MSL0025351

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP[b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total[a]		
Hale Texas Region 8 USA, 2013 (DP 1044)	2.07	0.37	Seed	<0.0025	<0.0025	<0.0050	170	Trt. 3 MSL0025351
10OK Washita Oklahoma	0.96	0.129	Seed	<0.0025	<0.0025	<0.0050	167	Trt. 2 MSL0025351
Region 8 USA, 2013 (DP 1044)	2.07	0.278	Seed	<0.0025	<0.0025	<0.0050	167	Trt. 3 MSL0025351
11TX Hockley Texas	0.96	0.132	Seed	<0.0025	<0.0025	<0.0050	183	Trt. 2 MSL0025351
Region 8 USA, 2013 (DP 1044)	2.07	0.293	Seed	<0.0025	<0.0025	<0.0050	183	Trt. 3 MSL0025351
12CA Tulare California	0.96	0.142	Seed	<0.0025	<0.0025	<0.0050	178	Trt. 2 MSL0025351
Region 10 USA, 2013 (DP 1044)	2.07	0.305	Seed	<0.0025	<0.0025	<0.0050	178	Trt. 3 MSL0025351
13CA Tulare California	0.96	0.142	Seed	<0.0025	<0.0025	<0.0050	155	Trt. 2 MSL0025351
Region 10 USA, 2013 (DP 1044)	2.07	0.305	Seed	<0.0025	<0.0025	<0.0050	155	Trt. 3 MSL0025351

a) Total mg/kg = (Tioxazafen mg/kg) + (Benzamidine mg/kg). Benzamidine is reported in Tioxazafen equivalents. Values below the LOQ of 0.0025 mg/kg are summed as 0.0025.

b) DAP = Interval in days between planting of treated seed and harvesting of commodity. DAP for individual replicates is listed after the average value.

Animal feed

Maize forage and stover

Table 88 Residues of tioxazafen and benzamidine in maize forage and stover from supervised field trials in USA after seed treatment of tioxazafen 48.7% w/w SC

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP [b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total [a]		
01PA Lehigh Pennsylvania Region 1 USA, 2012 (DKC53-78)	0.53	0.039	Forage	<0.0025	<0.0025	<0.0050	94	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	129	
	1.02	0.076	Forage	<0.0025	<0.0025	<0.0050	94	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	129	
	2.1	0.156	Forage	<0.0025	<0.0025	<0.0050	94	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	129	

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP [b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total [a]		
02NC Wayne North Carolina Region 2 USA, 2012 (DKC58-83)	0.53	0.032	Forage	<0.0025	<0.0025	<0.0050	96	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	134	
	1.04	0.068	Forage	<0.0025	<0.0025	<0.0050	96	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	134	
	2.14	0.132	Forage	<0.0025	<0.0025	<0.0050	96	Trt. 4 MSL0025349
			Stover	0.0054	<0.0025	0.0079	134	
03MI Ottawa Michigan Region 5 USA,2012 (DKC46-07)	0.51	0.036	Forage	<0.0025	<0.0025	<0.0050	103	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	138	
	1.03	0.072	Forage	<0.0025	<0.0025	<0.0050	103	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	138	
	2.05	0.143	Forage	<0.0025	<0.0025	<0.0050	103	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	138	
04IN Elkhart Indiana Region 5 USA,2012 (DKC53-78)	0.53	0.037	Forage	<0.0025	<0.0025	<0.0050	106	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	149	
	1.02	0.071	Forage	<0.0025	<0.0025	<0.0050	106	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	149	
	2.1	0.151	Forage	<0.0025	<0.0025	<0.0050	106	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	149	
05IL Clinton Illinois Region 5 USA, 2012 (DKC62-97)	0.54	0.039	Forage	<0.0025	<0.0025	<0.0050	93	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
	1.07	0.077	Forage	<0.0025	<0.0025	<0.0050	93	Trt. 3 MSL0025349
			Stover	<0.0025	0.0031	0.0056	154	
	2.26	0.164	Forage	<0.0025	<0.0025	<0.0050	93	Trt. 4 MSL0025349
			Stover	<0.0025	0.0045	0.007	154	
06IL Stark Illinois Region 5 USA, 2012 (DKC62-97)	0.54	0.044	Forage	<0.0025	<0.0025	<0.0050	91	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	144	
	1.07	0.087	Forage	<0.0025	<0.0025	<0.0050	91	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	144	
	2.26	0.184	Forage	<0.0025	<0.0025	<0.0050	91	Trt. 4 MSL0025349
			Stover	0.0109	<0.0025	0.0134	144	
07IL Carroll Illinois Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Forage	<0.0025	<0.0025	<0.0050	110	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
	1.04	0.079	Forage	<0.0025	<0.0025	<0.0050	110	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
	2.14	0.162	Forage	<0.0025	<0.0025	<0.0050	110	Trt. 4 MSL0025349

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP [b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total [a]		
			Stover	<0.0025	<0.0025	<0.0050	154	
08IL Ogle Illinois Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Forage	<0.0025	<0.0025	<0.0050	110	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
	1.04	0.079	Forage	<0.0025	<0.0025	<0.0050	110	Trt. 3 MSL0025349
			Stover	0.0027	<0.0025	0.0052	154	
	2.14	0.162	Forage	<0.0025	<0.0025	<0.0050	110	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
09IA Wapello Iowa Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Forage	<0.0025	<0.0025	<0.0050	111	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	146	
	1.04	0.079	Forage	0.0025	<0.0025	0.005	111	Trt. 3 MSL0025349
			Stover	0.0062	<0.0025	0.0087	146	
	2.14	0.163	Forage	<0.0025	<0.0025	<0.0050	111	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	146	Forage not an average
10IA Jefferson Iowa Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Forage	<0.0025	<0.0025	<0.0050	117	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	153	
	1.04	0.079	Forage	<0.0025	<0.0025	<0.0050	117	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	153	
	2.14	0.162	Forage	<0.0025	<0.0025	<0.0050	117	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	153	
11IA Clinton Iowa Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Forage	<0.0025	<0.0025	<0.0050	110	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
	1.04	0.079	Forage	<0.0025	<0.0025	<0.0050	110	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
	2.14	0.162	Forage	<0.0025	<0.0025	<0.0050	110	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
12IA Louisa Iowa Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Forage	<0.0025	<0.0025	<0.0050	94	Trt. 2 MSL0025349
			Stover	0.0037	<0.0025	0.0062	165	
	1.04	0.079	Forage	<0.0025	<0.0025	<0.0050	94	Trt. 3 MSL0025349
			Stover	0.0035	<0.0025	0.006	165	
	2.14	0.162	Forage	<0.0025	<0.0025	<0.0050	94	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	165	
13IA Greene Iowa	0.53	0.036	Forage	<0.0025	<0.0025	<0.0050	88	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	126	

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP [b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total [a]		
Region 5 USA, 2012 (DKC59-35)	1.06	0.073	Forage	0.0056	<0.0025	0.0081	88	Trt. 3 MSL0025349
			Stover	0.0036	<0.0025	0.0061	126	
	2.15	0.148	Forage	0.008	<0.0025	0.0105	88	Trt. 4 MSL0025349
			Stover	0.0061	0.0035	0.0095	126	
14MO Adair Missouri Region 5 USA, 2012 (DKC58-83)	0.53	0.04	Forage	<0.0025	<0.0025	<0.0050	90	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	128	
	1.04	0.079	Forage	<0.0025	<0.0025	<0.0050	90	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	128	
	2.14	0.162	Forage	<0.0025	<0.0025	<0.0050	90	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	128	
15NE York Nebraska Region 5 USA, 2012 (DKC59-35)	0.53	0.042	Forage	<0.0025	<0.0025	<0.0050	102	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	152	
	1.06	0.087	Forage	<0.0025	<0.0025	<0.0050	102	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	152	
	2.15	0.181	Forage	<0.0025	<0.0025	<0.0050	102	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	152	
16NE Polk Nebraska Region 5 USA, 2012 (DKC59-35)	0.53	0.035	Forage	<0.0025	<0.0025	<0.0050	88	Trt. 2 MSL0025349
			Stover	<0.0025	0.0033	0.0058	152	
	1.06	0.07	Forage	<0.0025	<0.0025	<0.0050	88	Trt. 3 MSL0025349
			Stover	<0.0025	0.0061	0.0086	152	
	2.15	0.145	Forage	<0.0025	<0.0025	<0.0050	88	Trt. 4 MSL0025349
			Stover	<0.0025	0.0043	0.0068	152	
17NE Fillmore Nebraska Region 5 USA, 2012 (DKC59-35)	0.53	0.041	Forage	<0.0025	<0.0025	<0.0050	96	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	134	
	1.06	0.081	Forage	<0.0025	<0.0025	<0.0050	96	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	134	
	2.15	0.169	Forage	<0.0025	<0.0025	<0.0050	96	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	134	
18MN Freeborn Minnesota Region 5 USA, 2012 (DKC46-07)	0.51	0.044	Forage	<0.0025	<0.0025	<0.0050	109	Trt. 2 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
	1.03	0.089	Forage	<0.0025	<0.0025	<0.0050	109	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
	2.05	0.177	Forage	<0.0025	<0.0025	<0.0050	109	Trt. 4 MSL0025349
			Stover	0.0029	<0.0025	0.0054	154	
19MN	0.51	0.044	Forage	<0.0025	<0.0025	<0.0050	109	Trt. 2 MSL0025349

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP [b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total [a]		
Steele Minnesota Region 5 USA, 2012 (DKC46-07)			Stover	<0.0025	<0.0025	<0.0050	154	
	1.03	0.089	Forage	<0.0025	<0.0025	<0.0050	109	Trt. 3 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
	2.05	0.177	Forage	<0.0025	<0.0025	<0.0050	109	Trt. 4 MSL0025349
			Stover	<0.0025	<0.0025	<0.0050	154	
20WI Walworth Wisconsin Region 5 USA, 2012 (DKC53-78)	0.53	0.039	Forage	<0.0025	<0.0025	<0.0050	104	Trt. 2 MSL0025349
			Stover	<0.0025	0.0036	0.0061	150	
	1.02	0.076	Forage	0.0028	<0.0025	0.0053	104	Trt. 3 MSL0025349
			Stover	0.0042	0.0056	0.0098	150	
	2.1	0.155	Forage	0.0033	<0.0025	0.0058	104	Trt. 4 MSL0025349
			Stover	<0.0025	0.0045	0.007	150	
21TX Tom Green Texas Region 6 USA, 2012 (DKC62-97)	0.54	0.042	Forage	<0.0025	<0.0025	<0.0050	86	Trt. 2 MSL0025349
			Stover	<0.0025	0.0034	0.0059	118	
	1.07	0.081	Forage	<0.0029	<0.0025	0.0054	86	Trt. 3 MSL0025349
			Stover	<0.0025	0.0045	0.007	118	
	2.26	0.173	Forage	<0.0025	<0.0025	<0.0050	86	Trt. 4 MSL0025349
			Stover	<0.0031	0.0049	0.008	118	
22OK Caddo Oklahoma Region 6 USA, 2012 (DKC59-35)	0.53	0.046	Forage	<0.0025	<0.0025	<0.0050	94	Trt. 2 MSL0025349
			Stover	<0.0025	0.0123	0.0148	115	
	1.06	0.093	Forage	<0.0025	0.003	0.0055	94	Trt. 3 MSL0025349
			Stover	<0.0032	0.0098	0.013	115	
	2.15	0.189	Forage	<0.0025	0.0026	0.0051	94	Trt. 4 MSL0025349
			Stover	<0.0028	0.0107	0.0135	115	

[a] Total mg/kg = (Tioxazafen mg/kg) + (Benzamidine mg/kg). Benzamidine is reported in Tioxazafen equivalents. Values below the LOQ of 0.0025 mg/kg are summed as 0.0025.

[b] DAP = Interval in days between planting of treated seed and harvesting of commodity. DAP for individual replicates is listed after the average value.

Soya bean forage and hay

Table 89 Residues of tioxazafen and benzamidine in soya bean forage and hay from supervised field trials in USA after seed treatment of tioxazafen SC

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP[b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total[a]		
01NC	0.57	0.244	Forage	0.0252	0.0147	0.0398	78	Trt. 2 MSL0025350
			Hay	0.0551	0.0622	0.1173	86	

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP[b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total[a]		
Wayne North Carolina Region 2 USA, 2013 (AG6132)								
	0.93	0.38	Forage	0.0339	0.0206	0.0544	78	Trt. 3 MSL0025350
			Hay	0.0756	0.0816	0.1572	86	
02SC Barnwell South Carolina Region 2 USA, 2013 (AG6132)	0.57	0.163	Forage	0.0208	0.0168	0.0375	51	Trt. 2 MSL0025350
			Hay	0.1151	0.0536	0.1687	59	
	0.93	0.266	Forage	0.0365	0.0158	0.0522	51	Trt. 3 MSL0025350
			Hay	0.04	0.0395	0.0795	59	
03AR Crittenden Arkansas Region 4 USA, 2013 (AG4832)	0.53	0.19	Forage	0.0278	0.0502	0.078	46	Trt. 2 MSL0025350
			Hay	0.0296	0.0658	0.0954	53	
	0.93	0.333	Forage	0.0362	0.0542	0.0904	46	Trt. 3 MSL0025350
			Hay	0.034	0.0788	0.1128	53	
04MO Butler Missouri Region 4 USA, 2013 (AG4832)	0.53	0.183	Forage	0.0106	0.0332	0.0437	50	Trt. 2 MSL0025350
			Hay	0.0213	0.0657	0.087	57	
	0.93	0.32	Forage	0.017	0.0418	0.0588	50	Trt. 3 MSL0025350
			Hay	0.0511	0.1075	0.1586	57	
05MS Washington Mississippi Region 4 USA, 2013 (AG4832)	0.53	0.17	Forage	0.0069	0.021	0.0279	46	Trt. 2 MSL0025350
			Hay	0.0313	0.0778	0.109	53	
	0.93	0.298	Forage	0.0134	0.0358	0.0492	46	Trt. 3 MSL0025350
			Hay	0.0742	0.1235	0.1977	53	
06LA Rapides Louisiana Region 4 USA, 2013 (AG4832)	0.53	0.257	Forage	0.0169	0.0274	0.0443	44	Trt. 2 MSL0025350
			Hay	0.0125	0.0644	0.0769	51	
	0.93	0.472	Forage	0.0242	0.0362	0.0603	44	Trt. 3 MSL0025350
			Hay	0.021	0.0961	0.117	51	
07MI Ottawa Michigan Region 5 USA, 2013 (AG2031)	0.54	0.173	Forage	0.0039	0.0072	0.0111	59	Trt. 2 MSL0025350
			Hay	0.0108	0.0156	0.0263	66	
	1.09	0.352	Forage	0.0078	0.008	0.0159	59	Trt. 3 MSL0025350
			Hay	0.0134	0.0182	0.0316	66	

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP[b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total[a]		
08IN Montgomery Indiana Region 5 USA, 2013 (AG4130)	0.54	0.222	Forage	0.0118	0.0188	0.0306	78	Trt. 2 MSL0025350
			Hay	0.0133	0.0316	0.0449	86	
	0.96	0.364	Forage	0.0081	0.0191	0.0272	78	Trt. 3 MSL0025350
			Hay	0.0214	0.0373	0.0587	86	
09IL Clinton Illinois Region 5 USA, 2013 (AG4130)	0.54	0.23	Forage	0.0048	0.0178	0.0226	41	Trt. 2 MSL0025350
			Hay	0.0085	0.0595	0.0679	48	
	0.96	0.355	Forage	0.0048	0.0189	0.0237	41	Trt. 3 MSL0025350
			Hay	0.012	0.0674	0.0793	48	
10IL Madison Illinois Region 5 USA, 2013 (AG4130)	0.54	0.208	Forage	0.0082	0.0298	0.038	39	Trt. 2 MSL0025350
			Hay	0.0116	0.112	0.1236	46	
	0.96	0.37	Forage	0.0116	0.0399	0.0515	39	Trt. 3 MSL0025350
			Hay	0.0139	0.1058	0.1196	46	
11IL Carroll Illinois Region 5 USA, 2013 (AG3130)	0.46	0.148	Forage	0.0034	0.0054	0.0088	52	Trt. 2 MSL0025350
			Hay	0.0135	0.0299	0.0434	58	
	0.88	0.284	Forage	0.0029	0.0066	0.0095	52	Trt. 3 MSL0025350
			Hay	0.0208	0.0413	0.0621	58	
12IA Jefferson Iowa Region 5 USA, 2013 (AG3130)	0.46	0.148	Forage	0.0026	0.0088	0.0114	56	Trt. 2 MSL0025350
			Hay	0.0055	0.0328	0.0382	62	
	0.88	0.192	Forage	0.0026	0.0099	0.0125	56	Trt. 3 MSL0025350
			Hay	0.0111	0.0432	0.0542	62	
13IA Wapello Iowa Region 5 USA, 2013 (AG3130)	0.46	0.148	Forage	0.0067	0.0138	0.0205	42	Trt. 2 MSL0025350
			Hay	0.0036	0.028	0.0316	48	
	0.88	0.284	Forage	0.0064	0.0186	0.025	42	Trt. 3 MSL0025350
			Hay	0.0082	0.0531	0.0613	48	
14IA Louisa Iowa Region 5 USA, 2013	0.46	0.148	Forage	0.0249	0.0495	0.0744	41	Trt. 2 MSL0025350
			Hay	0.0197	0.08	0.0997	47	
	0.88	0.284	Forage	0.0127	0.0342	0.0468	41	Trt. 3 MSL0025350

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP[b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total[a]		
(AG3130)								
			Hay	0.0208	0.078	0.0987	47	
15IA Clinton Iowa Region 5 USA, 2013 (AG3130)	0.46	0.148	Forage	0.0031	0.0068	0.0099	52	Trt. 2 MSL0025350
			Hay	0.011	0.0344	0.0453	58	
	0.88	0.284	Forage	0.0031	0.0078	0.0109	52	Trt. 3 MSL0025350
			Hay	0.0128	0.0454	0.0582	58	
16MO Adair Missouri Region 5 USA, 2013 (AG3130)	0.46	0.148	Forage	0.0468	0.019	0.0658	43	Trt. 2 MSL0025350
			Hay	0.0146	0.0554	0.07	49	
	0.88	0.284	Forage	0.025	0.0145	0.0395	43	Trt. 3 MSL0025350
			Hay	0.0315	0.0806	0.112	49	
17NE York Nebraska Region 5 USA, 2013 (AG3130)	0.46	0.196	Forage	<0.0025	0.0135	0.016	56	Trt. 2 MSL0025350
			Hay	0.0067	0.0376	0.0443	64	
	0.88	0.385	Forage	0.0055	0.015	0.0205	56	Trt. 3 MSL0025350
			Hay	0.0142	0.052	0.0662	64	
18NE Polk Nebraska Region 5 USA, 2013 (AG3130)	0.46	0.163	Forage	0.0113	0.0228	0.034	50	Trt. 2 MSL0025350
			Hay	0.0103	0.069	0.0792	57	
	0.88	0.322	Forage	0.0187	0.0316	0.0503	50	Trt. 3 MSL0025350
			Hay	0.024	0.1115	0.1355	57	
19NE Seward Nebraska Region 5 USA, 2013 (AG3130)	0.46	0.16	Forage	0.0082	0.0263	0.0345	46	Trt. 2 MSL0025350
			Hay	0.0089	0.0733	0.0822	53	
	0.88	0.308	Forage	0.0088	0.0352	0.044	46	Trt. 3 MSL0025350
			Hay	0.0098	0.0919	0.1016	53	
20MN Freeborn Minnesota Region 5 USA, 2013 (AG2031)	0.54	0.207	Forage	0.0028	0.0075	0.0103	54	Trt. 2 MSL0025350
			Hay	0.0096	0.0211	0.0307	61	
	1.09	0.42	Forage	0.0044	0.0065	0.0109	54	Trt. 3 MSL0025350
			Hay	0.009	0.0226	0.0316	61	
21MN Steele Minnesota	0.54	0.204	Forage	0.003	0.0099	0.0129	48	Trt. 2 MSL0025350
			Hay	0.0056	0.0258	0.0314	55	

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP[b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total[a]		
Region 5 USA, 2013 (AG2031)	1.09	0.418	Forage	0.0048	0.0096	0.0144	48	Trt. 3 MSL0025350
			Hay	0.0115	0.0327	0.0442	55	
22WI Walworth Wisconsin Region 5 USA, 2013 (AG2031)	0.54	0.227	Forage	0.0105	0.0145	0.0249	50	Trt. 2 MSL0025350
			Hay	0.0148	0.0446	0.0594	57	
	1.09	0.458	Forage	0.0105	0.0071	0.0176	50	Trt. 3 MSL0025350
			Hay	0.0133	0.0272	0.0405	57	

[a] Total mg/kg = (Tioxazafen mg/kg) + (Benzamidine mg/kg). Benzamidine is reported in Tioxazafen equivalents. Values below the LOQ of 0.0025 mg/kg are summed as 0.0025.

[b] DAP = Interval in days between planting of treated seed and harvesting of commodity. DAP for individual replicates is listed after the average value.

Cotton Gin by-products

Table 90 Residues of tioxazafen and benzamidine in cotton gin by-products from supervised field trials in the USA after seed treatment with tioxazafen 48.7% w/w SC

Trial code, Location, NAFTA Region, Country, years, (Variety)	Seed Treatment Rate		Portion Analysed	Residues (mg/kg)			DAP[b] (days)	Reference and Remarks
	mg ai/seed	kg ai/ha		Tioxazafen	Benzamidine	Total[a]		
08TX Tom Green, Texas Region 8 USA, 2013 (DP 1044)	0.96	0.11	Gin By-Products	<0.0025	0.0037	0.0062	151	Trt. 2 MSL0025351
	2.07	0.238	Gin By-Products	<0.0025	0.006	0.0085	151	Trt. 3 MSL0025351
09TX Hale Texas Region 8 USA, 2013 (DP 1044)	0.96	0.172	Gin By-Products	<0.0025	0.004	0.0065	170	Trt. 2 MSL0025351
	2.07	0.37	Gin By-Products	<0.0025	0.0089	0.0114	170	Trt. 3 MSL0025351
10OK Washita Oklahoma Region 8 USA, 2013 (DP 1044)	0.96	0.129	Gin By-Products	<0.0025	0.0027	0.0052	168	Trt. 2 MSL0025351
	2.07	0.278	Gin By-Products	<0.0025	0.003	0.0055	168	Trt. 3 MSL0025351
11TX Hockley Texas Region 8 USA, 2013 (DP 1044)	0.96	0.132	Gin By-Products	<0.0025	0.0073	0.0098	184	Trt. 2 MSL0025351
	2.07	0.293	Gin By-Products	<0.0025	0.0128	0.0153	184	Trt. 3 MSL0025351

[a] Total mg/kg = (Tioxazafen mg/kg) + (Benzamidine mg/kg). Benzamidine is reported in Tioxazafen equivalents. Values below the LOQ of 0.0025

mg/kg are summed as 0.0025.

[b] DAP = Interval in days between planting of treated seed and harvesting of commodity. DAP for individual replicates is listed after the average value.

FATE OF RESIDUES IN STORAGE AND PROCESSING

Maize

A processing study for maize after seed treatment with tioxazafen SC was conducted (Mueth, M.G., 2014, report No. MSL0025349, MRID 49304265). The maize was grown from seed treated at 2.1 mg/seed which is twice the maximum proposed seed treatment rate from tioxazafen. Two separate treated and untreated bulk maize grain samples were composited from maize collected from multiple locations from residue study for processing. The grain was processed into grits, meal, flour, and oil from dry processing, and starch and oil from wet processing. Processed samples were stored frozen after collection and were extracted for analysis within 63 days of sampling. The samples were analysed with methods AG-ME-1604 for tioxazafen and method AG- ME-1579 for benzamidine in maize RACs and processed fractions. The LOQs were validated to 0.0025 mg/kg for both tioxazafen and benzamidine in maize RACs and processed fractions. There were no residues of either tioxazafen or benzamidine above the LOQ (0.0025 mg/kg) in grain or any of the processed fractions. Concentration factors could not be determined for any of the processed fractions because there were no measurable residues of either tioxazafen or benzamidine in any fractions, including the grains used for processing.

Soya bean

A processing study for soya bean after seed treatment with tioxazafen SC was conducted in USA (Mueth, M.G., 2014, report No. MSL0025350, MRID 49304266). The soya bean was grown from seed treated at 1.0 mg/seed. Treated and untreated bulk soya bean seed samples were collected from two locations from the soya bean residue study for processing. The seed was processed into meal, hulls, crude oil, crude lecithin, toasted meal, degummed oil, and refined, bleached and deodorized (RBD) oil. The samples were analysed with methods AG-ME-1604 for tioxazafen and method AG- ME-1579 for benzamidine in soya bean RACs and processed fractions. The LOQs were validated to 0.0025 mg/kg for both tioxazafen and benzamidine in soya bean RACs and processed fractions.

Results are summarised in Tables 91 and 92. Processing of soya bean seed into meal, hulls, crude oil, crude lecithin, toasted meal, degummed oil and RBD oil showed that residues of benzamidine are limited to the meal and hull fractions. Meal (untoasted and toasted) had concentration factors for benzamidine of approximately 1.5 while hulls had a concentration factor of 0.12 to 0.70 in the two processed samples. There were no quantifiable parent tioxazafen residues in either the unprocessed seed or any of the processed fractions.

Table 91 Summary of Residues from Processing of Soya bean Seed after seed treatment of Tioxazafen 48.7% w/w SC at rate of 1.0 mg ai/seed

Processed Fraction	Average Residue [a] (mg/kg)				Concentration Factors, Benzamidine [bc]
	Site 06LA		Site 10IL		
	tioxazafen	Benzamidine	tioxazafen	Benzamidine	
Seed for processing	<0.0025	0.0422	<0.0025	0.0291	--
Meal	<0.0025	0.0560	<0.0025	0.0432	1.33, 1.49 (1.41)
Hulls	<0.0025	0.0052	<0.0025	0.0203	0.12, 0.70 (0.41)
Crude oil	<0.0025	<0.0025	<0.0025	<0.0025	0.06, 0.09 (0.08)
Crude lecithin	<0.0025	0.0033	<0.0025	<0.0025	0.08, 0.09 (0.09)
RBD oil	<0.0025	<0.0025	<0.0025	<0.0025	0.06, 0.09 (0.08)
Meal (toasted)	<0.0025	0.0640	<0.0025	0.0466	1.52, 1.60 (1.56)
Degummed oil	<0.0025	<0.0025	<0.0025	<0.0025	0.06, 0.09 (0.08)

[a] Residues are expressed as each analyte in tioxazafen equivalents, and are the site-averaged values for each location.

[b] Concentration factor = benzamidine residue in processed fraction/ benzamidine residue in corresponding seed. Concentration factors for each site are listed, with average in parentheses.

Table 92 Residues of tioxazafen and Benzamidine in Soya bean Processed Fractions after seed treatment of Tioxazafen 48.7% w/w SC at rate of 1.0 mg ai/seed

Site	Benzamidine Amount (mg/kg)		tioxazafen Amount (mg/kg)		Total (mg/kg)	
Processed Fraction	Individual	Site Average	Individual	Site Average	Site Average	Conc. Factor [a]
06LA (Rapides, Louisiana, USA, Region 4)						
Seed from Processor	0.0418	0.0422	<0.0025	<0.0025	0.0447	1
	0.0425		<0.0025			
Meal	0.0451	0.0560	<0.0025	<0.0025	0.0585	1.33
	0.0668		<0.0025			
Hulls	0.0052	0.0052	<0.0025	<0.0025	0.0077	0.12
	0.0052		<0.0025			
Crude Oil	<0.0025	<0.0025	<0.0025	<0.0025	<0.0050	0.06
	<0.0025		<0.0025			
Crude Lecithin	na[b]	0.0033	na[b]	<0.0025	0.0058	0.08
	0.0033		<0.0025			
RBD Oil	<0.0025	<0.0025	<0.0025	<0.0025	<0.0050	0.06
	<0.0025		<0.0025			
Meal (Toasted)	0.0639	0.0640	<0.0025	<0.0025	0.0665	1.52
	0.0640		<0.0025			
Degummed Oil	<0.0025	<0.0025	<0.0025	<0.0025	<0.0050	0.06
	<0.0025		<0.0025			
10IL (Madison, Illinois, USA, Region 5)						
Seed from Processor	0.0298	0.0291	<0.0025	<0.0025	0.0316	1
	0.0283		<0.0025			
Meal	0.0429	0.0432	<0.0025	<0.0025	0.0457	1.49
	0.0434		<0.0025			
Hulls	0.0200	0.0203	<0.0025	<0.0025	0.0228	0.70
	0.0206		<0.0025			
Crude Oil	<0.0025	<0.0025	<0.0025	<0.0025	<0.0050	0.09
	<0.0025		<0.0025			
Crude Lecithin	<0.0025	<0.0025	<0.0025	<0.0025	<0.0050	0.09
	<0.0025		<0.0025			
RBD Oil	<0.0025	<0.0025	<0.0025	<0.0025	<0.0050	0.09
	<0.0025		<0.0025			
Meal (Toasted)	0.0465	0.0466	<0.0025	<0.0025	0.0491	1.60
	0.0466		<0.0025			
Degummed Oil	<0.0025	<0.0025	<0.0025	<0.0025	<0.0050	0.09
	<0.0025		<0.0025			

[a] Because there are no measurable tioxazafen residues in seed or any processed fraction, the concentration factor is the ration of benzamidine residue in the processed fraction to benzamidine residue in unprocessed seed returned from processor.

[b] Not Analysed - Sample lost during analytical workup.

Cotton

A processing study was planned to conduct on cotton following seed treatment with 47.3 or 47.8% (w/w) tioxazafen SC at exaggerated rates of 2.0 mg/seed (Mueth, M.G., 2014, Report No. MSL0025351, MRID 49304267). Undelinted cotton seed commodities were collected at the normal harvest time. All cotton seed samples were extracted within 190 days after sampling. All samples were analysed within 4 days after extraction with method AG-ME-1604 for the analyses of tioxazafen or AG- ME-1579 for benzamidine. The LOQs of methods were validated to 0.0025 mg/kg for both tioxazafen and benzamidine in cotton RACs. Benzamidine levels are expressed in tioxazafen equivalents. As the application rate was limited by the ability to load tioxazafen onto the cotton seeds, and thus the exaggerated rate (2 mg/seed) was only twice the maximum intended seed treatment rate, rather than the 5X rate specified in the processing guideline. As there were no residues above the LOQ (0.0025 mg/kg) in cotton undelinted seed, samples were not processed and processing study was not conducted.

LIVESTOCK FEEDING STUDIES

Lactating dairy cow

The Meeting received the information on a lactating dairy cow feeding study (Brungardt, 2014, MSL0025801). 18 Holstein dairy cows were dosed orally by capsules fortified with Tioxazafen once daily for 28 consecutive days. The 18 cows were divided into five treatment groups (control, 1×, 5×, 25×, and 100×) equal to 0ppm, 0.12 ppm (1×), 0.60 ppm (5×), 3.00 ppm (25×) and 12.0 ppm (100×) in feed, corresponded to 0.0041, 0.020, 0.096 and 0.396 mg/kg bw/day.

Milk was collected twice daily during the dosing period and composited by day. Milk samples from all dose groups were analysed for one or more tioxazafen metabolite residues on Days -1, 1, 4, 7, 10, 13, 16, 19, 22, 25, and 28. Additionally, a portion of the Day 22, 25, and 28 milk samples from the control, 25×, and 100× treatment groups were separated into skim milk and cream for separate analyses. On Day 29, one cow from the control group, all cows in the 1×, 5×, and 25× dose groups, and three cows from the 100× dose group were sacrificed and liver, kidney, composite muscle, subcutaneous fat, mesenterial fat, and perirenal fat were collected for analysis. The remaining cows entered into a 10-day depuration phase with periodic collection and analysis of milk and tissues.

All matrices were analysed using method ME-1764-03 for both tioxazafen and benzamidine. Milk, liver and kidney were also analysed for 2-thenoylglycine, and fat was also analysed for benzonitrile. Residues of tioxazafen in milk, skim milk, cream, liver, kidney and muscle, and residues of tioxazafen and benzonitrile in subcutaneous fat, mesenterial fat, and perirenal fat were quantitated by electron impact gas chromatography/tandem mass spectrometry (EI GC/MS/MS). Residues of benzamidine in muscle and subcutaneous fat, mesenterial fat and perirenal fat, and residues of benzamidine and 2-thenoylglycine in milk, skim milk, cream, liver and kidney were quantitated by high performance liquid chromatography/electrospray ionisation/tandem mass spectrometry (LC/MS/MS). The residues of all metabolites are reported as tioxazafen equivalents (molecular weight ratios: tioxazafen /benzamidine=1.90; tioxazafen /2-thenoylglycine=1.23; tioxazafen /benzonitrile=2.21). The LOQ and limit of detection (LOD) for tioxazafen, benzamidine, 2-thenoylglycine, and benzonitrile are presented in Table 93.

Table 93 LODs and LOQs of tioxazafen and Metabolites in Cattle Milk and Tissues

Matrix	Tioxazafen (mg/kg)		Benzamidine (mg/kg)[a]		2-Thenoylglycine (mg/kg)[a]		Benzonitrile (mg/kg)[a]	
	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD
Milk	0.010	0.0031	0.010	0.0024	0.010	0.0017	—[b]	—
Liver	0.010	0.0020	0.010	0.0022	0.060	0.0091	—	—
Kidney	0.010	0.0015	0.010	0.0017	0.025	0.0049	—	—
Muscle	0.010	0.0014	0.010	0.0015	—	—	—	—
Fat	0.010	0.0027	0.010	0.0008	—	—	0.025	0.0036

[a] Reported as tioxazafen equivalents

[b] Not applicable

There was no apparent negative effect on feed consumption and milk production by treatment with tioxazafen. Residues in milk and tissues from cows dosed with tioxazafen at levels of 1×, 5×, 25×, and 100× for 28 days are shown in Table 94. Residues in whole milk, skim milk, and cream from cows dosed with tioxazafen at levels of 25 and 100 for 22, 25, and 28 days are shown in Table 95.

Table 94 Residues of tioxazafen in Milk and Tissues from Cows Dosed for 28 Days

Cattle Matrix	Analyte	Residues by Feeding Level (mg/kg)[a]				
		Control	0.12ppm (1×)	0.60ppm (5×)	3.00ppm (25×)	12.0ppm (100×)
Milk						
	Tioxazafen	<0.010 [40][b]	ND[c]	ND	ND	<0.010 [60]
	Benzamidine	<0.010 [44]	<0.010 [30]	<0.010 [30]	Av 0.0234 [30] (0.0266)	Av 0.0676 [60] (0.0801)
	2-Thenoylglycine	<0.010 [42]	ND	ND	<0.010 [30]	Av 0.0146 [60] (0.0178)
Liver						
	Tioxazafen	<0.010 [2]	ND	ND	ND	<0.010 [3]
				0.0185 0.0118	0.0541 0.0416	0.104 0.125

Cattle Matrix	Analyte	Residues by Feeding Level (mg/kg)[a]				
		Control	0.12ppm (1×)	0.60ppm (5×)	3.00ppm (25×)	12.0ppm (100×)
	Benzamidine	<0.010 [3]	<0.010 [3]	0.0126 Av 0.0143 (0.0185)	0.0461 Av 0.0473 (0.0541)	0.163 Av 0.131 (0.163)
	2-Thenoylglycine	<0.060 [3]	ND	ND	ND	<0.060 [3]
Kidney						
	Tioxazafen	<0.010 [2]	ND	ND	ND	<0.010 [3]
	Benzamidine	<0.010 [3]	<0.010 [3]	0.0177 0.0140 0.0132 Av 0.0150 (0.0177)	0.0594 0.0668 0.0688 Av 0.0650 (0.0688)	0.194 0.194 0.135 Av 0.174 (0.194)
	2-Thenoylglycine	<0.025 [3]	<0.025 [3]	<0.025 [3]	0.0484 0.0460 0.0408 Av 0.0451 (0.0484)	0.1170 0.1130 0.0882 Av 0.106 (0.1170)
Muscle						
	Tioxazafen	<0.010 [2]	ND	ND	ND	<0.010 [3]
	Benzamidine	<0.010 [3]	<0.010 [3]	<0.010 [3]	0.0141 0.0101 0.0112 Av 0.0118 (0.0141)	0.0334 0.0243 0.0410 Av 0.0329 (0.0410)
Subcutaneous Fat						
	Tioxazafen	<0.010 [2]	ND	ND	ND	<0.010 [3]
	Benzamidine	<0.010 [3]	<0.010 [3]	<0.010 [3]	<0.010 [3]	0.0160 0.0113 0.0143 Av 0.0139 (0.0160)
	Benzonitrile	<0.025 [2]	ND	ND	ND	<0.025 [3]
Mesenterial Fat						
	Tioxazafen	<0.010 [2]	ND	ND	ND	<0.010 [3]
	Benzamidine	<0.010 [3]	<0.010 [3]	<0.010 [3]	<0.010 [3]	0.0171 0.0120 0.0071[d] Av 0.0121 (0.0171)
	Benzonitrile	<0.025 [2]	ND	ND	ND	<0.025 [3]
Perirenal Fat						
	Tioxazafen	<0.010 [2]	ND	ND	ND	<0.010 [3]
	Benzamidine	<0.010 [2]	<0.010 [3]	<0.010 [3]	0.0130 0.0061[d] 0.0179 Av 0.0123 (0.0179)	0.0382 0.0240 0.0495 Av 0.0372 (0.0495)
	Benzonitrile	<0.025 [2]	ND	ND	ND	<0.025 [3]

[a] The overall average is listed for milk, with the maximum daily average in parentheses. Individual values and the overall average 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; in such cases, the number of samples analysed follows in brackets. The residues of all metabolites are reported as tioxazafen equivalents.

[b] Value in brackets [ ] indicates the number of replicate samples with a given residue value. For milk residues above the LOQ, the value in brackets indicates the number of samples included in the average value.

[c] ND = Not determined (samples not analysed because samples in higher dose group had residues <LOQ).

[d] Benzamidine residues in fat that were below the LOQ but above the LOD. Values included in averages.

Table 95 Average Daily Residues of tioxazafen in Milk, Skim Milk, and Cream from Cows Dosed for 28 Days

Dose group	Matrix	Day	Tioxazafen (mg/kg)[a]	Benzamidine (mg/kg)[a]	2-Thenoylglycine (mg/kg)[a]
12.0 mg/kg (100×)	Milk	22	<0.010	0.0604	0.0140
		25	<0.010	0.0600	0.0134
		28	<0.010	0.0678	0.0127
	Skim Milk	22	<0.010	0.0664	0.0155
		25	<0.010	0.0703	0.0147
		28	<0.010	0.0640	0.0138
	Cream	22	<0.010	0.0574	0.0128
		25	<0.010	0.0596	0.0121
		28	<0.010	0.0544	0.0116
3.00 mg/kg (25×)	Milk	22	ND[b]	0.0242	<0.010
		25	ND	0.0230	<0.010
		28	ND	0.0235	<0.010
	Skim Milk	22	ND	0.0213	<0.010
		25	ND	0.0207	<0.010
		28	ND	0.0245	<0.010
	Cream	22	ND	0.0194	<0.010
		25	ND	0.0187	<0.010
		28	ND	0.0225	<0.010

[a] Milk values are averages of six animals, and skim milk and cream values are averages of three animals. Values below the LOQ are listed as <0.010. The residues of all metabolites are reported as tioxazafen equivalents.

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

There were no quantifiable residues of tioxazafen in any of the milk samples from the 100× dose group, and most samples had no detectable residues. No detectable residues of tioxazafen were found in skim milk or cream samples from the 100× dose group.

Maximum benzamidine residues in milk samples from the 100×, 25×, 5× and 1× dose levels were 0.106 mg/kg, 0.0350 mg/kg, <LOQ, and <LOQ, respectively. Maximum 2-thenoylglycine residues in the milk samples from the 100× and 25× dose groups were 0.0304 mg/kg and <0.010 mg/kg (LOQ), respectively.

In the six animals from the 100× dose group, the average residue levels of benzamidine in milk reached a plateau at 4 days and varied between an average residue of 0.0600 mg/kg and 0.0801 mg/kg for the remainder of the samples until the dosing ceased. Average residue levels of 2-thenoylglycine in milk also reached a plateau at 4 days and varied between an average residue of 0.0127 mg/kg and 0.0178 mg/kg for the remainder of the samples until dosing ceased.

For the 25× dose group, the average residue levels of benzamidine in milk also reached a plateau at 4 days and varied between an average residue of 0.0209 mg/kg and 0.0266 mg/kg for the remainder of the samples until the dosing ceased. Average residue levels of 2-thenoylglycine in milk for the 25× dose group remained below the LOQ at all sampling times.

The concentration factors for benzamidine and 2-thenoylglycine residues for skim milk and cream were determined (in the 100× and 25× dose groups for Days 22, 25, and 28) by dividing the average residue value for each sampling day by the average residue value of the corresponding milk samples. The concentration factors for benzamidine were 0.88 to 1.15 in skim milk and 0.77 to 0.99 in cream. For 2-thenoylglycine, concentration factors were 0.93 to 1.05 in skim milk and 0.80 to 0.94 in cream, showing no significant concentration of residues for either metabolite in skim milk or cream.

For the cattle tissue samples, no detectable residues of tioxazafen were found in liver samples and fat samples from the 100× dose group. Residues in kidney and muscle tissues remained below the LOQ of 0.010 mg/kg.

All tissue samples (liver, kidney, muscle, and fat) from the 100× dose group had benzamidine residues close to or above the LOQ. The highest residues were in kidney and liver, with average residues for the 100× dose group of 0.174 mg/kg and 0.131 mg/kg, respectively. Residues above the LOQ were found for several matrices in the lower dose groups, but in the 1× dose group, benzamidine residues in all tissues were <LOQ.

Liver and kidney samples were also analysed for 2-thenoylglycine. In liver, all samples from the 100× dose group were <LOQ. In kidney, the residues from the 100×dose group were all above the LOQ with an average residue of 0.106 mg/kg. All kidney samples in the 5× and 1× dose groups had 2-thenoylglycine residues <LOQ.

All three types of fat samples were also analysed for benzonitrile. Average benzonitrile residues in fat in the 100× dose group were detectable but <LOQ.

In addition to the primary tissue samples taken after sacrifice on Day 29, depuration samples were taken on Days 31, 34, and 38. Residues in milk and tissues from the depuration phase are shown in table 96 and **Error! Reference source not found.** 97, respectively.

Table 96 Residues of tioxazafen in Milk from Cows in the 100× Treatment Group from Final Dose through Depuration Phase

Summary of Residues in Milk From the 100× Treatment Group (average mg/kg)			
Sampling Interval (Days)	Tioxazafen	Benzamidine [a]	2-Thenoylglycine [a]
28[b]	<0.010	0.0678	0.0127
30[b]	<0.010	<0.010	<0.010
33[c]	<0.010	<0.010	<0.010
37[d]	<0.010	<0.010	<0.010

[a] Reported as tioxazafen equivalents.

[b] Average of six animals.

[c] Average of three animals.

[d] Average of two animals.

Table 97 Residues of tioxazafen in Tissues from Cows in the 100× Treatment Group from Final Dose through Depuration Phase

Summary of Residues in Tissues From the 100× Treatment Group (average mg/kg)							
Analyte	Day	Liver	Kidney	Muscle	Subcutaneous Fat	Mesenterial Fat	Perirenal Fat
Tioxazafen	29[a]	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
	31[b]	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
	34[b]	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
	38[b]	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzamidine[c]	29[a]	0.131	0.174	0.0329	0.0139	0.0121	0.0372
	31[b]	0.0143	0.0199	<0.010	<0.010	<0.010	<0.010
	34[b]	0.0118	<0.010	<0.010	<0.010	<0.010	<0.010
	38[b]	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
2-Thenoylglycine[c]	29[a]	<0.060	0.106	ND[d]	ND	ND	ND
	31[b]	<0.060	<0.025	ND	ND	ND	ND
	34[b]	<0.060	<0.025	ND	ND	ND	ND
	38[b]	<0.060	<0.025	ND	ND	ND	ND
Benzonitrile[c]	29[a]	ND	ND	ND	<0.025	<0.025	<0.025
	31[b]	ND	ND	ND	<0.025	<0.025	<0.025
	34[b]	ND	ND	ND	<0.025	<0.025	<0.025
	38[b]	ND	ND	ND	<0.025	<0.025	<0.025

[a] Average of three animals.

[b] Single values.

[c] Reported as tioxazafen equivalents.

[d] ND = Not determined (samples not analysed because samples in higher dose group had residues <LOQ).

Residues of benzamidine and 2-thenoylglycine declined rapidly in milk collected during the depuration period with residues below the LOQ within 2 days after dosing stopped. Residues of all analytes were below the LOQ by Day 38.

The maximum daily average residues during the dosing period (for milk), and the maximum and average residues at sacrifice within 24 hours after the last dose (tissues) are summarised in table 98.

Table 98 Summary of Maximum and Average Residues of tioxazafen in Cattle Commodities by Feeding Level during and Directly after Dosing

Analyte / Cattle Matrix	Maximum (Average) Residues by Feeding Level[a] (mg/kg)			
	0.12 mg/kg (1×)	0.60 mg/kg (5×)	3.00 mg/kg (25×)	12.0 mg/kg (100×)
Tioxazafen				
Milk	ND[b]	ND	ND	<0.010[c]
Liver	ND	ND	ND	<0.010
Kidney	ND	ND	ND	<0.010
Muscle	ND	ND	ND	<0.010
Fat[d]	ND	ND	ND	<0.010
Benzamidine				
Milk	<0.010	<0.010	0.0266	0.0801
Liver	<0.010	0.0185 (0.0143)	0.0541 (0.0473)	0.163 (0.131)
Kidney	<0.010	0.0177 (0.0150)	0.0688 (0.0650)	0.194 (0.174)
Muscle	<0.010	<0.010	0.0141 (0.0118)	0.0410 (0.0329)
Fat[d]	<0.010	0.0114 (<0.010)	0.0179 (<0.010)	0.0495 (0.0211)
2-Thenoylglycine				
Milk	ND	ND	<0.010	0.0178
Liver	ND	ND	ND	<0.060
Kidney	<0.025	<0.025	0.0484 (0.0451)	0.117 (0.0106)
Benzonitrile				
Fat[d]	ND	ND	ND	<0.025

[a] Maximum daily average for milk. Maximum individual value for all other tissues, with dose group average in parentheses. The residues of all metabolites 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.

Table 99 Residue Levels of tioxazafen and its metabolites in High Dose (12 mg/kg in the Diet) Milk Samples from the Dairy Cow Feeding Study

Sampling Day/Group	Sample ID	Tioxazafen (mg/kg)[a]	Benzamidine (mg/kg)[a,b]	2-Thenoylglycine (mg/kg)[a,b]
Milk	LOD:	0.0031	0.0024	0.0017
100× Treated Dairy Cows (average 12.0 mg/kg in feed)				
-1 Day 100×	Average	<LOD	<LOD	[0.0033]
1 Day 100×	Average	<LOD	0.0514	0.0119
4 Day 100×	Average	<LOD	0.0719	0.0148
7 Day 100×	Average	<LOD	0.0693	0.0151
10 Day 100×	Average	[<0.0034]	0.0801	0.0178
13 Day 100×	Average	[<0.0031]	0.0702	0.0151
16 Day 100×	Average	[<0.0033]	0.0696	0.0153

Sampling Day/Group	Sample ID	Tioxazafen (mg/kg)[a]	Benzamidine (mg/kg)[a,b]	2-Thenoylglycine (mg/kg)[a,b]
Milk	LOD:	0.0031	0.0024	0.0017
19 Day 100×	Average	<LOD	0.0751	0.0156
22 Day 100×	Average	<LOD	0.0604	0.0140
25 Day 100×	Average	<LOD	0.0600	0.0134
28 Day 100×	Average	<LOD	0.0678	0.0127

[a] Individual analyte residues <LOD are reported as <LOD; averages are calculated using the value equal to the LOD. Residue values >LOD, but <LOQ are presented in brackets. ND= not determined.

[b] Values are reported in parent equivalents (molecular weight ratios: tioxazafen/benzamidine=1.90. tioxazafen/2-thenoylglycine=1.23).

The LOQ is 0.010 mg/kg for tioxazafen, benzamidine and 2-thenoylglycine in milk.

Laying hens

A residue feeding study (Brungardt J.N., 2014, report No. MSL0025802, MRID 49304263) was conducted in laying hens with Tioxazafen to determine the residues of tioxazafen and its metabolites benzamidine and benzonitrile in eggs and tissues from hens dosed with tioxazafen. Tioxazafen was fed orally by capsule to 72 laying hens in single daily oral doses.

The 72 hens were divided into five groups (control, 1×, 5×, 25×, and 100×). The 1 dose level (0.80 ppm in feed) is an exaggerated dose level compared to anticipated poultry dietary exposure levels (dietary burdens) resulting from the proposed uses of tioxazafen as a seed treatment on crops (maize, soya bean, and cotton). The 1× dose level and the 100-fold range of dose levels were selected based on transfer factors determined from the metabolism study of tioxazafen in laying hens. The actual dose rates achieved in the study for the treated animals were 0.81 ppm in feed (0.055 mg/kg bw/day, 1×), 4.0 ppm in feed (0.26 mg/kg bw/day, 5×), 20.8 ppm in feed (1.34 mg/kg bw/day, 25×), and 79.1 ppm in feed (5.24 mg/kg bw/day, 100×).

The hens were dosed daily for 28 days. Egg samples from all dose groups were analysed on Days -1, 1, 4, 7, 10, 13, 16, 19, 22, 25, and 28. Additionally, on Days 21, 24, and 27, eggs collected from the control group, 25× dose group, and 100× dose group were separated into egg yolk and egg white fractions for separate analyses. After the dosing on Day 28, four hens from the control group, all hens in the 1×, 5×, and 25× groups, and 12 hens from the 100× dose group were sacrificed and liver, muscle, and fat samples were collected. The remaining hens entered into a 10-day depuration phase with periodic collection and analysis of eggs and tissues. Within each treatment group, egg and tissue samples from each subgroup (consisting of four hens) were composited prior to analysis.

All matrices were analysed for both tioxazafen and benzamidine. Fat was also analysed for benzonitrile. The maximum daily average residues during the dosing period (for eggs), and the maximum and average residues at sacrifice within approximately 8.5 hours after the last dose (tissues) are summarised in table 100.

The hens were observed at least once daily for any abnormalities from the start of the acclimation period until the conclusion of the in-life phase of the study. On Day 17 one hen from the 100× group was found dead and on Day 20 one hen from the 5× group was found dead. Both hens died as a result of a prolapsed oviduct. All other hens were observed to be healthy and normal throughout the acclimation and dosing phase of the study, the weights were not adversely affected by treatment with tioxazafen.

Residues in eggs and tissues from chickens dosed with tioxazafen at levels of 1×, 5×, 25×, and 100× for 28 days are summarised in Table 101.

Table 100 Residue Levels of tioxazafen and its metabolites in High Dose Egg samples from the laying hen Feeding Study

Sampling Day/Group	Sample ID	Tioxazafen (mg/kg)[a]	Benzamidine (mg/kg)[a,b]
Egg	LOD	0.0034	0.0036
100× Treated Laying Hens (average 79.1ppm in feed)			
-1 Day 100×	Average	<LOD	<LOD
1 Day 100×	Average	<LOD	<LOD
4 Day 100×	Average	[0.0053]	[0.0090]
7 Day 100×	Average	0.0112	0.0177
10 Day 100×	Average	0.0117	0.0169
13 Day 100×	Average	0.012	0.0165
16 Day 100×	Average	0.0131	0.0168

Sampling Day/Group	Sample ID	Tioxazafen (mg/kg)[a]	Benzamidine (mg/kg)[a,b]
19 Day 100×	Average	0.0135	0.0167
22 Day 100×	Average	0.014	0.0178
25 Day 100×	Average	0.018	0.0239
28 Day 100×	Average	0.0176	0.0229
25× Treated Laying Hens (average 20.8 ppm in feed)			
-1 Day 25×	Average	<LOD	<LOD
4 Day 25×	Average	<LOD	[0.0043]
7 Day 25×	Average	<LOD	[0.0074]
10 Day 25×	Average	<LOD	[0.0067]
13 Day 25×	Average	<LOD	[0.0066]
16 Day 25×	Average	[<0.0035]	[0.0075]
19 Day 25×	Average	[0.0037]	[0.0080]
22 Day 25×	Average	<LOD	[0.0073]
25 Day 25×	Average	<LOD	[0.0081]
28 Day 25×	Average	[0.0034]	[0.0083]
5× Treated Laying Hens (average 4.0 ppm in feed)			
-1 Day 5×	Average	<LOD	<LOD
19 Day 5×	Average	ND	<LOD
1× Treated Laying Hens (average 0.81 ppm in feed)			
-1 Day 1×	Average	<LOD	<LOD

[a] Individual analyte residues <LOD are reported as <LOD; averages are calculated using the value equal to the LOD. Residue values >LOD, but <LOQ are presented in brackets. ND= not determined.

[b] Values are reported in parent equivalents (molecular weight ratios: Tioxazafen/benzamidine=1.90.)

The LOQ is 0.010 mg/kg for Tioxazafen and benzamidine in eggs.

Table 101 Residues of tioxazafen in Eggs and Tissues from Hens Dosed for 28 Days

Poultry Matrix	Analyte	Residues by Feeding Level (mg/kg)[a]				
		Control	0.81 ppm (1×)	4.0 ppm (5×)	20.8 ppm(25×)	79.1 ppm (100×)
Egg						
	Tioxazafen	<0.010 [41][b]	ND[c]	ND	<0.010 [27]	Av 0.0120 [60] (0.0239)
	Benzamidine	<0.010 [42]	ND	<0.010 [3]	<0.010 [27][d]	Av 0.0162 [60] (0.0273)
Liver						
	Tioxazafen	<0.010 [4]	<0.010 [3]	<0.010 [3]	<0.010 [3]	<0.010 [3]
	Benzamidine	<0.010 [5]	<0.010 [3]	0.0122 0.0134 0.0148 Av 0.0135 (0.0148)	0.0787 0.0744 0.0612 Av 0.0714 (0.0787)	1.03 0.785 0.606 Av 0.807 (1.03)
Muscle						
	Tioxazafen	<0.010 [3]	ND	ND	ND	<0.010 [3]
	Benzamidine	<0.010 [4]	<0.010 [3]	<0.010 [3]	<0.010 [3]	0.0175 0.0177 0.0176 Av 0.0176 (0.0177)
Fat						
	Tioxazafen	<0.010 [5]	<0.010 [3]	0.0106 <0.010 <0.010 Av <0.010 (0.0106)	0.0445 0.0361 0.0519 Av 0.0442 (0.0519)	0.287 0.362 0.327 Av 0.325 (0.362)
	Benzamidine	<0.010 [5]	ND	ND	ND	<0.010 [3]

Poultry Matrix	Analyte	Residues by Feeding Level (mg/kg)[a]				
		Control	0.81 ppm (1×)	4.0 ppm (5×)	20.8 ppm(25×)	79.1 ppm (100×)
	Benzonitrile	<0.025 [5]	<0.025 [3]	<0.025 [3]	<0.025 [3]	0.0491 0.0645 0.0563 Av 0.0566 (0.0645)

[a] The overall average is listed for egg, with the maximum daily average in parentheses. Individual values and the overall average 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; in such cases, the number of samples analysed follows in brackets. Residues of benzamidine and benzonitrile are reported as tioxazafen equivalents.

[b] Value in brackets [ ] indicates the number of replicate samples with a given residue value. For egg residues above the LOQ, the value in brackets indicates the number of samples included in the average value.

[c] ND = Not determined -samples not analysed because samples in higher dose group had residues <LOQ

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

Table 102 Average Daily Residues of tioxazafen in Egg White and Egg Yolk from Hens Dosed for 28 Days

Dose group	Matrix	Day	tioxazafen(mg/kg)[a]	Benzamidine (mg/kg)[a]
79.1 mg/kg (100× ⍰)	Whole Egg	22	0.0140	0.0178
		25	0.0180	0.0239
		28	0.0176	0.0229
	Egg White	21	<0.010	<0.010
		24	<0.010	<0.010
		27	<0.010	<0.010
	Egg Yolk	21	0.0465	0.0495
		24	0.0517	0.0624
		27	0.0636	0.0704
20.8 mg/kg (25× ⍰)	Whole Egg	22	<0.010	<0.010
		25	<0.010	<0.010
		28	<0.010	<0.010
	Egg White	21	ND[b]	ND
		24	ND	ND
		27	ND	ND
	Egg Yolk	21	<0.010	0.0169
		24	0.0106	0.0224
		27	<0.010	0.0226

[a] All values are averages of nine samples. Values below the LOQ are listed as <0.010. Residues of benzamidine and benzonitrile are reported as tioxazafen equivalents.

[b] ND = not determined (samples not analysed because samples in higher dose group had residues <LOQ).

The Day 22, 25 and 28 whole egg samples are included for comparison with the Day 21, 24 and 27 separated white and yolk samples.

Maximum tioxazafen residues in egg samples from the 100× and 25× dose groups were 0.0239 mg/kg and <LOQ, respectively. Maximum benzamidine residues in egg samples from the 100× and 25× dose groups were 0.0273 mg/kg and 0.0111 mg/kg, respectively. No detectable residues of benzamidine were found in egg samples from the 5 dose group.

The average daily residue levels of parent tioxazafen in eggs from the 100× dose group increased through Day 7, then leveled off and varied between an average residue of 0.0112 mg/kg and 0.0180 mg/kg for the remainder of the samples until the dosing ceased. Average residue levels of benzamidine in eggs also increased through Day 7 and varied between an average residue of 0.0165 mg/kg and 0.0239 mg/kg for the remainder of the samples until dosing ceased. With both analytes, the residues reached

a maximum by Day 25. By the end of the depuration phase (10 days after the last dose; Day 38), the residues in eggs dropped to below the LOD.

For the 25× dose group, the average residue levels of tioxazafen in eggs were primarily below the LOD, with only a few samples slightly above the LOD. The residue levels of benzamidine in eggs were higher but remained below the LOQ at all times.

Residues were determined for egg whites and egg yolks from egg samples collected from the 100× and 25× dose groups on Days 21, 24, and 27. Average residues of tioxazafen ranged from 0.0465 to 0.0636 mg/kg while benzamidine residues ranged from 0.0495 to 0.0704 mg/kg in egg yolks from the 100× dose group. No residues of either tioxazafen or benzamidine were found in any of the egg white samples from the 100× dose group. For egg yolks from the 25× dose group, average tioxazafen residues ranged from 0.0088 to 0.0106 mg/kg while benzamidine residues ranged from 0.0169 to 0.0226 mg/kg. Concentration factors were determined for tioxazafen and benzamidine in egg yolks and are summarised in table 103.

Table 103 Concentration Factors in Eggs

Matrix	Sampling Day / Group	Tioxazafen[a]	Benzamidine[a]
Egg Yolk	21 Day 100×	3.04	2.64
	24 Day 100×	2.58	2.46
	27 Day 100×	3.48	2.92
Egg Yolk	21 Day 25×	NA[c]	2.32[b]
	24 Day 25×	NA[c]	2.77[b]
	27 Day 25×	NA[c]	2.72[b]

[a] Concentration factors were calculated using the average residue value of the egg yolks divided by the average residue value of the corresponding whole egg samples (Days 22, 25, and 28). Numerical values between LOD and LOQ (not listed) were used in the calculation as needed.

[b] Based on benzamidine residue values in whole egg that were below the LOQ.

[c] NA = Not applicable. Concentration factors for egg yolks could not be calculated for tioxazafen because residue values in the corresponding whole egg samples were <LOD.

For the tissue samples, no quantifiable residues of tioxazafenwere found in liver and muscle samples from the 100 dose group and no detectable residues were found in liver samples from the 25×, 5×, or 1× dose groups. Residues in fat samples averaged 0.325 mg/kg, 0.0442 mg/kg, <LOQ, and <LOQ for the 100×, 25×, 5×, and 1× dose groups, respectively.

Liver, muscle, and fat samples were also analysed for benzamidine. In liver, maximum benzamidine residues from the 100×, 25×, 5× and 1× dose groups were 1.03 mg/kg, 0.0787 mg/kg, 0.0148 mg/kg, and <LOQ, respectively. Maximum benzamidine residues in muscle samples from the 100× dose group were 0.0177 mg/kg. There were no quantifiable residues of benzamidine in muscle samples from the 25×, 5×, and 1× dose groups. There were no quantifiable residues of benzamidine in fat samples from the 100 dose group.

Fat samples were also analysed for benzonitrile. Maximum benzonitrile residues in fat samples from the 100× dose group were 0.0645 mg/kg. There were no quantifiable residues of benzonitrile in fat samples from the 25×, 5×, and 1× dose groups.

In addition to the primary tissue samples taken after sacrifice on Day 28, depuration samples were taken on Days 31, 34, and 38. Residues in eggs and tissues from the depuration phase are shown in Table 104 and Table 105, respectively.

Table 104 Residues of tioxazafen in Eggs from Hens in the 100× Treatment Group from Final Dose through Depuration Phase

Summary of Residues in Eggs (average mg/kg)		
Sampling Interval (Days)	Tioxazafen	Benzamidine[a]
28[b]	0.0176	0.0229
31[c]	0.0127	0.0165
34[d]	<0.010	<0.010
38[e]	<0.010	<0.010

[a] Residues of benzamidine reported as tioxazafen equivalents.

[b] Average of six subgroups of hens.

[c] Average of three subgroups of hens.

[d] Average of two subgroups of hens.

[e] Single values.

Table 105 Residues of tioxazafen in Tissues from Hens in the 100× Treatment Group from Final Dose through Depuration Phase

Summary of Residues in Tissues From the 100× Treatment Group (average mg/kg)				
Analyte	Day	Liver	Muscle	Fat
Tioxazafen	28[a]	<0.010	<0.010	0.325
	31[b]	<0.010	<0.010	0.0159
	34[b]	<0.010	<0.010	<0.010
	38[b]	<0.010	<0.010	<0.010
Benzamidine[c]	28[a]	0.807	0.0176	<0.010
	31[b]	0.191	<0.010	<0.010
	34[b]	0.0982	<0.010	<0.010
	38[b]	0.0649	<0.010	<0.010
Benzonitrile[c]	28[a]	ND[d]	ND	<0.025
	31[b]	ND	ND	<0.025
	34[b]	ND	ND	<0.025
	38[b]	ND	ND	<0.025

[a] Average of three subgroups of hens.

[b] Single values.

[c] Residues of benzamidine and benzonitrile are reported as tioxazafen equivalents.

[d] ND = Not determined (samples not analysed or maximum residues for all samples within dose group were <LOQ).

By Day 38, residues of tioxazafen in all egg and tissue samples were below the LOQ. Residues of benzamidine were below the LOQ by Day 38 for egg, muscle, and fat samples. Liver had a residue value of 0.0649 mg/kg on Day 38. Residues of benzonitrile in fat were below the LOD by Day 38.

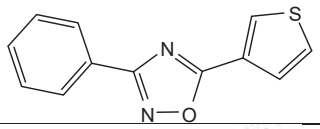
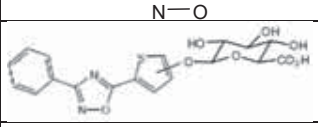
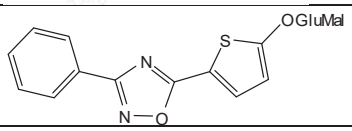
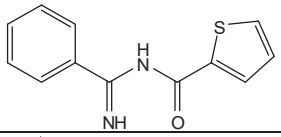
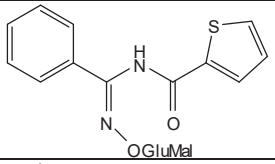
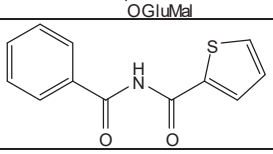
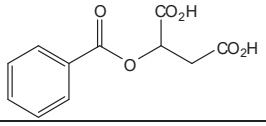
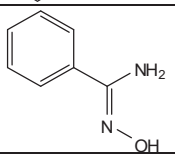
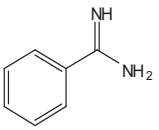
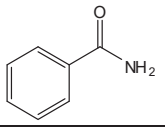
APPRAISAL

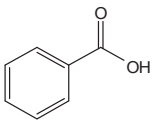
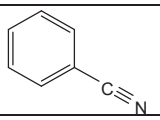
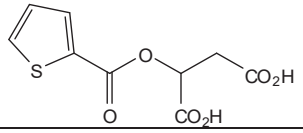
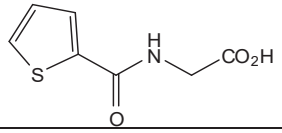
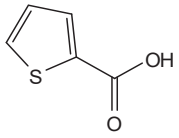
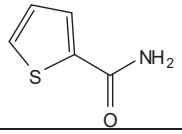
Tioxazafen is a seed treatment nematicide to control a broad-spectrum of nematodes in maize, soya bean, and cotton. Tioxazafen is a disubstituted oxadiazole, which represents a new class of nematicidal chemistry demonstrating activity against soya bean cyst, root knot and reniform nematodes in soya bean;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

Trivial Name	Chemical Name	Structure	Where Found
Benzoic acid	benzoic acid		Maize foliage, cotton foliage, rotational crops; Hen excreta, goat liver, goat skim milk anaerobic soil
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-3-13C, phenyl-U-14C]-tioxazafen (PH-T) or [oxadiazole-5-13C, thiophene-2-14C]-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 below 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.

Commodity		Recommended maximum residue levels (mg/kg)	STMR or STMR-P, median residue (mg/kg)	HR, HR-P, highest residue (mg/kg)
CCN	Name			
AB 1204	Cotton gin trash	0.02	Median: 0.00635 (as)	Highest: 0.0098 (as)
SO 0691	Cottonseed	0.01*	0	-
MO 0105	Edible offal (mammalian)	0.03	0.01	0.025
PE 0112	Eggs	0.02*	0	0.02
GC 0645	Maize	0.01*	0	-
AS 0645	Maize fodder	0.03(DM)	Median: 0.005 (as)	Highest: 0.013 (as)
MF 0100	Mammalian fats (except milk fats)	0.03	0.01	0.025
MM 0095	Meat (from mammals other than marine mammals)	0.02	0.01	0.02
ML 0106	Milks	0.02	0.01	-
PO 0111	Poultry edible offal	0.02*	0.02	0.02
PF 0111	Poultry fat	0.02*	0.01	0.02
PM 0110	Poultry meat	0.02*	0.01	0.02
VD0541	Soya bean (dry)	0.04	0.0125	-

Commodity		Recommended maximum residue levels (mg/kg)	STMR or STMR-P, median residue (mg/kg)	HR, HR-P, highest residue (mg/kg)
CCN	Name			
AL 0541	Soya bean fodder	0.4(DM)	Median: 0.069 (as)	Highest: 0.17 (as)
AB 1265	Soya bean meal	0.06	0.017	-

For dietary exposure and animal burden calculations

Commodity		Recommended maximum residue levels (mg/kg)	STMR or STMR-P, median residue (mg/kg)	HR, HR-P, highest residue (mg/kg)
CCN	Name			
OR 0541	Soya bean Refined oil		0	
AF 0645	Maize forage		0.005	0.0081
	Soya bean hull		0.0049	
AL 1265	Soya bean forage		0.033	0.078

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.

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The annual Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues was held in Berlin, Germany, from 18 to 27 September 2018. The FAO Panel of Experts had met in preparatory sessions from 13 to 17 September 2018. The Meeting was held in pursuance of recommendations made by previous Meetings and accepted by the governing bodies of FAO and WHO that studies should be undertaken jointly by experts to evaluate possible hazards to humans arising from the occurrence of pesticide residues in foods. During the meeting the FAO Panel of Experts was responsible for reviewing pesticide use patterns (use of good agricultural practices), data on the chemistry and composition of the pesticides and methods of analysis for pesticide residues and for estimating the maximum residue levels that might occur as a result of the use of the pesticides according to good agricultural use practices. The WHO Core Assessment Group was responsible for reviewing toxicological and related data and for estimating, where possible and appropriate, acceptable daily intakes (ADIs) and acute reference doses (ARfDs) of the pesticides for humans. This report contains information on ADIs, ARfDs, maximum residue levels, and general principles for the evaluation of pesticides. The recommendations of the Joint Meeting, including further research and information, are proposed for use by Member governments of the respective agencies and other interested parties.

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